ON GREEN ORGANISMS OCCURRING IN THE LOWER TROPOSPHERE

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(With Tab. III and IV).

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STATEMENT OF THE PROBLEM.

"Who can trace the aerial course of the spore?!"
Lange, Myc. 226, p. 1. 1934.

Microbiological investigations of the atmosphere have been carried out either from a general biological point of view or in the interest of hygiene, phytopathology, the study of allergic phenomena and entomology.

The Leyden Botanical Laboratory is interested in these studies chiefly from the point of view of geographical distribution of living beings.

As we know the universal distribution, not only of many fungi (in the broadest sense) but of protozoa and algae, mosses and ferns etc, as well; it should be of great interest to demonstrate the presence of the living state of such organisms, capable of active development, in the atmosphere.

Nobody has tried, as far as we are aware, to culture algae, mosses and ferns from atmospheric air collected by airplane (Chapter I).

A sampling apparatus, in which air is filtered through sterile absorbent materials, and which may be handled by simple methods from the cockpit of a plane was devised and constructed.

First of all the most suitable absorbent material was sought for and the filtration capacity of air through various absorbent materials was investigated in the wind tunnel at the Laboratory for Aerodynamics at Delft.

The wind tunnel was placed at our disposal for yet another experiment. In order to ascertain whether the closed compartments of the apparatus remained sterile in an air current a suspension of spores of *Penicillum glaucum* was blown into the tunnel.

The apparatus was finally mounted under the wing of an airplane. Before the sampling experiments were started a control flight was made up to 5000 M in order to ascertain whether the results would become unreliable by infection from the ground or from the plane.

Sampling flights were made up to 2000 M; one compartment was always left closed (Chapter II).

The sampling apparatus was returned to the laboratory after each flight. The absorbent material (glass wool) of each compartment was transferred to different nutrient media (Chapter III), in order to obtain various organisms (Chapter IV). A comparison of results with meteorological observations of the days previous to the experiment and at the time of the sampling flight seemed important. The atmospheric conditions were determined at different altitudes (Chapter V).

CHAPTER I.

Review and Discussion of the Literature.

Review of the Literature [1]).

The discoverer of the "little animals" VAN LEEUWENHOEK 1676 (26a and b) wrote to the Royal Society of London in several letters about the presence of living creatures in rain-, well-, sea- and snow water. The first letter on this subject is dated October 9th 1676. This letter was published in the Philosophical Transactions (41) of March 1677. "The rain water, which has been lifted up by the movement of the sun and has made the clouds, is mixed with the seed of little animals".

The consequences of van Leeuwenhoeks discovery did not fail to come and exerted their influence primarily on the medical world.

In the same volume of the "Transactions" we find;

"Some considerations of an observing person in the country upon Numb. 133 of these Transactions, sent in a letter to the Publisher of May 2, 1677". This "person" states;

"Mr. Leeuwenhoeks Microscopical Discoveries are exceeding curious, and may prompt us to suspect that our air is also vermiculated, and perhaps most of all in long calms, longlasting Eastern Winds, or much moisture in Spring-time, and in seasons of general Infections of Men or Animals....

And if we may be certain of seasons of great danger, I think we may be certain of effectual Remedies, by Gods blessing: ....And to interrupt the Calms and other annouances of the Air; we may apply all the helps recommended in Muffet's Improvement of Health, c. 4. viz. by noise of Bells, Guns, Drums, Trumpets, Tabrets and other Musical Instruments: by the chearful shouts of the people, and by cleansing all our Towns and Villages by Fire and pure Water, which will be more effectful, if it be done every where at the same set time, as when the Festival Bonefires were in use all over the Kingdom".

EHRENBERG (15) noticed in 1830 the objects in dust, especially in red coloured dust and in other blood-like phenomena.

He examined a dust-sample collected by DARWIN (12) 1831 when on board the Beagle near Porto Praya and found in it

[1]) As the literature on aerial organisms is very vast it would be outside the scope of this study to even attempt a survey of the most important articles. We shall confine ourselves, therefore, only to a few citations. Mr. T. Loosjes, former librarian of the Botanical Institute at Leyden has gathered a large number of titles which are at the disposal of those interested in this topic.

Infusoria, Diatomaceae and silicous tissues of plants.

The classical research of PASTEUR (39) 1861 upon spontaneous generation has convinced us that the air carries life, be it in latent or in vegetative form. Measured quantities of air were passed through gun cotton and the cotton dissolved afterwards in alcohol and ether. Pasteur found different microbes in the sediment. Their number depends considerably upon the locality and upon the elevation.

A second method used by Pasteur in examining the air content consists of exposing evacuated and sealed flasks containing a culture medium to the air by breaking the sealed tip. Pasteurs description of these flasks follows; "les ballons étaient primitivement vides d'air et remplis au tiers d'eau de levûre de bière, filtrée a limpidité parfaite".

After removal of the tip the flask is sealed by heat. Pasteur found the following results in examining the air in this manner at different altitudes up to 2000 M. quite near la Mer de Glace.

"Des vingt ballons ouverts dans la campagne, huit renferment des productions organisées; des vingt ballons ouverts sur le Jura, cinq seulement en contiennent; et enfin des vingt ballons remplis au Montanvert, par un vent assez fort, soufflant des gorges les plus profondes du glacier des Bois, un seul est altéré. Il faudrait sans doute multiplier beaucoup ces expériences. Mais telles qu'elles sont, elles tendent à prouver déjà qu'à mesure que l'on s'élève, le nombre des germes en suspension dans l'air diminue notablement. Elles montrent surtout la pureté, au point de vue qui nous occupe, de l'air des hautes cimes couvertes de glace, puisqu'un seul des vases remplis au Montanvert a donné naissance à une mucédinée".

With these results Pasteur furnished the proof, that not the air itself but the solid particles in the air are the cause of infection.

MIQUEL (35) gave, in 1883, a summary of the work done on microbial contents of the atmosphere which demonstrates the existence of viable eggs and spores. The greater part of the examinations cited by him were carried out in the interest of hygiene. Miquel especially stipulates the importance of this abundance of germs to man, and stresses the desirability of further study, especially of the pathogenic microbes.

He also confirmed Pasteurs results in so far as to show that the number of microbes isolated from the top of the Panthéon were less than the number isolated from "street-air" in the Centre of Paris.

BEIJERINCK (5a and b) found, in 1884, in rain drops on his window several salt crystals and andesite-like minerals doubtlessly belonging to the ashes ejected in the Krakatau eruption of 1883 and concluded from this fast dispersal of volcanic ashes that a similar dispersal of light and small organisms might be possible.

The "floating condition of life" in the air has been designated by MOLISCH (37) 1916 as the *aeroplankton.* He gives a clear exposure on the germ content of the atmosphere and its influence on mankind; he mentioned the former experimentators. Molisch used for the examination of air both petri-dishes with nutrient media, which were exposed 5—15' and afterwards incubated, and slides smeared with glycerol.

Those slides were brought at several locations inside and outside the town and showed afterwards soot, linen, wool, cotton, plant-hairs, pollen, diatoms, spores etc.

1. *Description of methods used on the ground.*

Aeroplankton has been investigated from several angles and by different methods.

Several authors have given a summary or description of methods.

MIQUEL 1883 and PETRI (40) 1888 mentioned older literature. Miquel describes aeroscopes used by Pouchet, Maddox, Cunningham, Schoenauer and Yung.

PAUL LINDNER (28) 1909 investigated the air in breweries. Glass-cylinders were exposed to the air during an hour. The spores were covered by nutrient gelatine as a so-called "roll-culture" after sedimentation in the cylinder.

The Committee on Standard methods for the Examination of air (11) has given in 1910, 1913, 1917, several reports in the interest of hygiene. Now the Standard method is an air filtration used by Petri. Air is conducted through a tube with 1 cm of sand as absorbent material. The sand is shaken in sterile water; this water is transferred to petri-dishes with nutrient agar.

A detailed historical summary has been given by RUEHLE (48) 1915. He pointed at the errors made by counting bacterial colonies in petri-dishes, which have been exposed to the air, because one dust particle may contain several microbes. Ruehle discussed the Standard Aeroscope, a modification of the Standard Aeroscope and also Rettgers Aeroscope.

The Standard Aeroscope consists of a glass tube closed at each end by a rubber stopper through which a thin glass tube

is passed. Inside the rubber stopper a thin layer of cotton-wool is placed on which 1 cm of sand is put as absorbent material. A physiological salt solution filters the air in Rettgers Aeroscope.

LODE (30) and VON ANGERER (1) 1929 both give reviews on the method of the examination of air during several years. Lode mentions, amongst other methods, Owen's Dustcounter, a simple apparatus, which is based upon the sedimentation of dust in a definite quantity of air.

Von Angerer devided the examination of air in a physiological and a biological part.

In the "physiological part", including three methods; sedimentation, filtration and a combination of the two, the air has to be freed from particles as completely as possible; the "biological part" consists in the cultivation of the organisms.

A summary of absorbent material used by different investigators follows;

sand, asbestos fibre, cotton-wool, glass-wool, powdered sugar, water, glass dust etc.

Almost every author discusses the different results in relation to location and meteorological conditions.

2. *Qualitative work.*

Only a restricted group, chiefly heterotrophic, of the air flora has been generally investigated.

The greater number of the publications deals with bacteria, fungi, yeasts chiefly in the interest of hygiene, phytopathology or the study of allergic phenomena.

Air samples collected in late years by airplane or balloon have been examined from the same point of view.

MIQUEL 1883, MOLISCH 1916, LODE and VON ANGERER 1929 reviewed the nineteenth and early 20th century hygienic literature.

Especially the extensive and exact examinations of SAITO (49a, b, c and d) 1904, 1906, 1908, 1922 ought to be mentioned at this place.

Saito studied the frequency of germs in the air of streets, houses and gardens during various seasons. He cultured fungi, yeasts and bacteria and demonstrated their frequency to be dependent upon both locality and meteorological condition.

PUSCHKAREW (45) 1913 was the only investigator who cultured protozoa from the air.

In 1910, at the instigation of Bütschli Puschkarew started to investigate dust-samples, but he soon rejected this method as

unreliable because of the possibility of previous infection by living beings [2]).

He chose other methods and exposed 3—25 days to the air a sterile dish with a sterilised culture solution, the dish being closed by gauze. A skin was formed after some days containing bacteria, fungi and protozoa.

He also used rainwater, collected by means of a funnel in a sterile flask, to which a culture medium was added.

Furthermore he used an aeroscope with cotton wool through which air was sucked by means of an air pump with a capacity of 390 L/m.

His results do not convincingly prove the cosmopolitan distribution of protozoa. In 1.9% of the air samples (fresh water) protozoa appeared.

According to Puschkarew it is impossible that a wind of average velocity would be able to raise the zoogloea from the substrate, only ascending air columns of high velocity such as occur in hurricanes and are known to be able even to disperse fishes and frogs would be able to cause the distribution of protozoa.

Unfortunately Puschkarew only used a single culture solution, of which he does not give the composition.

3. *Investigations on various localities.*

As mentioned before the results of the experiments depend on both location and elevation.

A large amount of research work has been performed in or at a short distance from towns.

Amongst others Galli Valerio (18) 1910 examined mountain-air on germ-content and compared these results with the air in rooms occupied by men or animals.

Hesse (22) 1913 and Bisby (6) 1935 studied the aeroplankton above the sea and found a very small number of bacteria and fungi. Hesse found even less germs in the air of the arctic zones.

The air has been also investigated above woods and deserts by Bonnier 1911 and Brown 1930 (8).

Prát (42) 1925 found on petri-dishes with nutrient agar exposed to the air in a stalactite cave near Jasov (Slovakia) *Sarcina* and *Micrococcus.*

In the course of time the balloon and the airplane have come to our aid in the study of the aeroplankton. The apparatus used

[2]) The same objection was raised by Puschkarew against the use of dust-samples by Ehrenberg.

for experiments from balloons or airplanes are usually different from the apparatus used for sampling air near the groundlevel with the aid of a pump.

a. *Investigation at high altitudes with the aid of a balloon.*

The first person who sampled from a balloon was CHRISTIANI (10) in 1893. He collected air samples above Geneva at an altitude of 1700 meters.

FLEMMING (17) 1908 found germs in the air up till 4000 meters, he mentioned an injurious influence of solar radiation and noticed the greatest number of organisms under clouds.

HARZ (cited by Flemming) obtained similar results in 1903.

HAHN (20) 1909 repeated these investigations and noticed a cloudlike dispersal of bacteria in the air. The germ content seemed to go parallel with the quantity of dust.

CHATTERJEE (9) 1931 used unmanned balloons to which a sampling-apparatus was attached. The apparatus consisted of an enclosed slide with an adhesive surface, which may be closed by the burning of a fuse. A second fuse brought the balloon down, which could be sent back to the author for a reward.

Chatterjee does not mention his results.

STEVENS and ROGERS (53) 1936 used a large sterilized tube with an adhesive surface, which could be opened and closed mechanically by means of cotton-wool stoppers. This tube was dropped from the gondola at 71000 ft. (21640 meters) altitude.

From air between 71000 ft.—35000 ft. (21640—10670 meters) ten different species of bacteria and molds have been grown.

b. *Investigations at high altitudes with the aid of the airplane.*

It is only recently that the airplane has made it possible to examine the aeroplankton at different altitudes and in various regions.

STAKMAN, HENRY, CURRAN, CHRISTOPHER (52) 1923 have been pioneers in this field. They investigated the distribution of spores of pathogenic fungi by wind. In their first work an ordinary microscope-slide smeared lightly with vaseline on one side was exposed. Later they used a slide attached to a stick which was placed inside a bottle and which could be drawn from the bottle and exposed and replaced in the bottle after use.

Finally they exposed the slides mechanically. A "spore trap" was made with six compartments each containing a slide and fastened on the wing of a plane. The compartments could be opened and closed by pulling a cable.

"Spore traps" were exposed at several places over the Mississippi Valley.

"Spores and pollengrains were relatively abundant at altitudes up to 11000 ft. (3350 meters). Scarce at higher altitudes". No meteorological data are given bij these investigators.

MISCHUSTIN (36) 1926 collected the air over Moscow and made quantitative and qualitative experiments. The air flow through his collecting-device was tested in the Laboratory of Aerodynamics at Moscow.

The apparatus, an enclosed petri-dish fastened on a stick, was extended by the pilot. Mischustin found 5 bacteria/Liter at altitudes beneath 1000 meters.

GRAIGIE and POPP 1928 examined the air in Canada. Their publication is only known to us in the form of a citation by Proctor (44a).

BROWN (8) 1930 collected micro-organisms in the air of the arid Southwest with sterilised agar dishes and spore traps. Exposures were made for two minutes at a time. Fungi and bacteria were found and tested for pathogenicity. Plant pathogens are common, while also bacteria and other micro-organisms are abundantly present.

TALMAN (54) 1932 used an airplane and exposed glass microscope slides covered with a very thin coating of vaseline to determine the height to which the spores of the black stem rust occur in the American Spring-wheat area. It has been found that they extend up to a height of about 10.000 feet (3050 meters) and it is evident that regions lying far from infected areas may receive spores from these great altitudes.

Agar-plate collections were made in 1932 by MEIER, STEVENSON and CHARLES (34) on five flights over the Eastern United States at altitudes of 500 to 18.000 ft. (150—5490 meters), spores of pathogenic fungi were found to be widely distributed.

MEIER and LINDBERGH (33) 1935 showed the presence of spores and pollen over water and ice in Denmark, Iceland, Greenland and N. America.

This collection is the first of its kind at higher altitudes over northern lands.

As collecting-device the "Sky-Hook" was used. A cartridge containing a slide with an adhesive surface was fastened in an aluminum tubing, which projected vertically to a height approximately 2 ft. (60 centimeters) above the edge of the cockpit. The entire operation of exposing and closing the slide may be carried out by means of a handle.

Long exposures of thirty to sixty minutes were made. Above Greenland 70 different objects were found, a.o. asci of certain fungi, unicellular algae, fragments of filamentous algae and insect wings, diatoms, volcanic ash etc.

Counts and photographs are given in their paper but no cultures were made.

Meier and Lindbergh found the arctic air more thinly populated with micro-organisms than that over the continents in more temperate regions.

Proctor (44a and b) 1934, 1935 made collections of air samples in the vicinity of Boston at different altitudes. On the first flights agar plates were exposed but as danger of contamination from extraneous sources could not be excluded in this way, a simple mechanism was constructed as follows;

A plate with twelve holes, six free and six with paper filters (of which the paper was saturated with a hydrocarbon oil), may be rotated in a cylindrical brass compartment with an inlet- and an outlet-tube.

The entire apparatus was sterilised previous to the flight.

Proctor states:

"Certain control flights were made to insure as far as possible, that such bacteria as were obtained came from the air at the levels indicated". He does not explain how he obtained this certainty. Tests made in the Technology wind-tunnel indicated an air-flow through the device of 1 cubic foot per minute (28,3 L/m) under general flight conditions.

One dish remained unexposed at each experiment. The paper dishes were examined with a microscope under sterile precautions and shaken in tubes with 10 cc of sterile water afterwards. The content of each tube was divided over three or four petri-plates to which agar was added afterwards.

Bacteria and molds were found above 19.600 ft. (5970 meters).

Yeasts and pollen were found above 16000 ft. (4880 meters).

Dust counts were made; a decrease was found at higher altitudes.

Durham (14) 1935 studied the geographic distribution of the pollen of hay-fever plants and their relative importance in allergy and examined 13000 oil-coated slides from 70 cities.

In September 1931 exposures were made from airplanes at various levels up to 4000 feet (12200 meters) over the land northwest of North Chicago and at the same time over the lake, 30 miles east of North Chicago.

Over the land the bulk of the pollen was below 1500 ft.

(457 meters) over the lake, the heavy concentration was found at 2000 ft. (610 meters) with a separate pollen cloud at 100 to 500 feet (30—152 meters).

MAC GUIDDY (31) 1935 used petri-dishes connected by means of a stick which he extended outside the plane. He found pollen above Omaha up to 3000 ft. (915 meters) and a decrease of bacteria above 4000 ft. (1220 meters).

The altitude record: 28.800 ft. (8780 meters) was established by WALKER (57) in 1935.

His method however was insufficient and showed the necessity of mechanical means for opening, exposing and closing of the sample-apparatus.

Walker used petri-dishes with nutrient agar, which were exposed by hand during ½—1 minute outside the plane. His hands became cold and numb at altitudes of 28.500 ft. (8690 meters) with a temperature of -34° F., the medium too being frozen. He found the atmosphere to be sterile between 20.000 to 28.800 ft. (6095—8780 meters). (Compare, however, the results of the balloonists Stevens and Rogers (53) pg. 395).

In a lecture for the London School of Hygienic and Tropical Medical Science MAC LEAN (32) 1935 gave a short summary of the investigations about the aeroplankton. He summarized as follows: "Micro-organisms have been found by means of air planes at an altitude up to 20.000 ft. (6095 meters) in sporadic clouds. A multiplication of these little beings should be possible under the clouds, which gives rise to a cloud-flora. Many of these germs are protected against ultra-violet rays by red and orange pigments and get heat by absorption of the thermic rays. Formaldehyde and radio-active substances in the air might have a stimulating influence on the growth of such little objects".

These rather fantastic statements need no further comment.

The aeroplankton at higher altitudes has been investigated by BERLAND (4a and b) 1933, 1934 COLLINS and BAKER 1934 from an entomological point of view.

They mentioned the American entomologist M. Coad, who designed complicated traps and sampled at altitudes between 500 to 5000 meters.

Berland used a net made of pure silk with thin meshes, which was fixed on a plane and tested in the wind-tunnel on strength.

The results were unexpected, an enormous fauna of small insects was observed decreasing at higher altitudes. Besides these animals, that floated in the air and were carried off by thermic winds and air-currents, he found material of botanical

and mineralogical nature. The quantity depended upon the season and locality.

4. *Dispersal of lower organisms.*

Most of the investigators mentioned above were struck by the possibilities for dispersal, especially of the lower organisms. These organisms appear to be able to endure the most unfavourable conditions and they may be carried moreover for hundreds of miles in horizontal direction and also move in vertical direction by means of ascending air-currents.

In an article published in the Scientific Monthly of January 1935 MEIER states;

"The potentialities of world-wide distribution of spores of fungi and other organisms caught up and carried abroad by trans-continental winds may be of tremendous economic consequence".

a. Work of a general nature.

RIDLEY (47) gave in 1930 a comprehensive summary of the manner in which plant-dispersal may be brought about. He emphasized the part played by wind in covering the earth with vegetation during the first epochs in evolution. This first vegetation consisted according to Ridley of *Filicinae, Bryophyta, Algae, Equisetaceae, Lycopodiaceae* next to *Bacteria, Fungi, Yeasts* and *Actinomycetes.*

A similar process occurs whenever a new island arises. Ridley showed that the cosmopolitic nature of the lower plants is not only due to their larger possibility for dispersal, but also to the fact that their requirements are few in relation to their milieu.

The *Myxomycetes* e.g. are ubiquitous not only by the easy dispersal of their spores by wind but also by their simple requirements.

The greater part of Ridley's work deals with the dispersal of higher plants.

With respect to the dispersal of seeds, spores of ferns, mosses and algae he states that little is known about the altitudes which might be reached. The remarks of HERZOG (21) on the dispersal of mosses are of interest in this connection.

GRÖNBLAD (19) mentioned in 1933 the British algologist Ralf, who demonstrated the presence in the atmosphere of myriads of spores of *Desmids* and lower Cryptogams, that develop in a suitable culture medium.

According to LANGE (25) 1934 the difference between European and American fungus-flora is not as great as may be expected

from American Monographs and Flora's. A newly "described" species of a Danish *Sphagnum* was found in Oregon near the Pacific Coast. Lange states; "Who can trace the aerial course of the spore?!"

ERNST (16) 1934 pointed at the great importance of the wind in the dispersal of plants and animals especially with respect to the "Krakatau problem". He gives a summary of the authors, who theorized upon the possibilities of dispersal of flora and fauna by water, wind, birds and men.

He only mentioned the dispersal in the upper air of fungi and cites as sole authority Stakman c.s.

A summary of the plant-geographical literature of the lower organisms is to be found in the work of BAAS BECKING (2) 1934.

He mentioned in relation to the transport of germs through the atmosphere the appearence of volcanic ash in rain water studied by Beijerinck.

Beijerinck found, in 1884, in raindrops volcanic ash from the Krakatau eruption of 1883 (see before pg. 392).

Baas Becking estimated the period in which a protozoal-cyst remains in the stratosphere as 2—6 months. A descent of this spore near the antipodes is therefore not improbable.

His calculation was based upon a method used by Humphreys for volcanic ash. Among others Walter, Entz and Mrazek are mentioned.

Walter described bacteria, fungi and plankton-algae as cosmopolitan. Géza Entz explained in 1884 the presence of the marine protozoa population of the Salt Lakes in Siebenbürgen by wind-transport. Mrazek 1902 found in hot-houses the fauna, which may be found only in similar conditions.

Baas Becking observed in 1932 in a newly established saltpool near Boekeloo (in the East of the Netherlands) several organisms specific for saline environment and known from other parts of the earth. It is possible to develop a specific microbial world, which is present everywhere, be it in latent or in active form, by using Beijerincks accumulation-methods. As there are scarce and less scarce organisms, it is not necessary to prescribe a certain area for an organism, just because it does not appear in a certain medium, but it is possible that the medium in that case was not sufficiently specific or that the organism occurred with a low frequency.

Two rules may be derived from Beijerincks work, as far as the microbes are concerned; "*Everything is everywhere*" and, from this "everything", "*the milieu selects*".

MAC LEAN (32) also emphasized the necessity of the use of varied culture media and upon lower incubation times, as many planktonic-organisms are very delicate.

b. Dust and dust-rain.

DARWIN gave, in his log book on board the Beagle, an elaborate account of atmospheric-dust. He collected an air sample which was examined by Ehrenberg. Moreover four airsamples collected by Lyell a hundred miles northward were investigated by Ehrenberg. The greater part of this dust samples contained infusoria and silicous tissues of plants (see above).

Darwin described this dust-rain as follows:

"The dust is of a brown colour, and under the blowpipe easily fuses into a black enamel. It is produced as I believe, from the wear and tear of volcanic rocks, and must come from the coast of Africa".

He concluded to a vast dispersal of the light and small spores of Cryptogams by means of these dust-rains.

BERKELEY 1857 [3]) pointed to the influence of winds, which carry spores of fungi mixed with dust thousands of miles before they are deposited.

There is a great deal of literature giving calculations of the vertical velocity and definite distances made by the aeroplankton.

SCHMIDT (51) 1925 calculated the velocities and altitudes attained by different botanical objects with fixed wind-velocity. The possibility of cosmopolitan dispersal of *Lycoperdon* (Gasteromycetes) and *Polytrichum* (Musci) is apparent from his numbers.

Pollen of Conifers may come down as a so-called "sulphur-rain". His theory was tested on a vessel 30 K.M. from the shore, where 162.000—88.000 pollengrains/square millimeters were deposited in May and June 1918. He calculated that only one pollen-grain out of 100.000 grains should reach an altitude of 3000 M and that organisms with a limited floating capacity have a scanty chance for dispersal in calm air.

The investigation of dust rains has been the topic of several researches.

We already mentioned EHRENBERGS work of 1830. He gave a summary of the studies about red dust and red rain and also described his own experiences.

As for the cause of this coloured material which was looked upon in olden times as divine warnings Ehrenberg was able, in many cases, to trace its origin. He found in the dust excrements of organisms, lower plants and animals and, moreover, among the

[3]) cited by Stevens, Meier and Lindbergh.

particles of the red dust certain green algae; *Chlorococcum, Protococcus* and *Vaucheria.*

According to Puschkarew Ehrenberg made an error in catching dust from roofs and other places, that might be previously infected by animals.

Also MOLISCH mentioned the common occurrence of dust rains of various colours, that contain as a rule *Diatoms,* plant tissues, seeds etc. next to inorganic substances. He referred further to the so-called."Kartoffelregen", when tubers of *Ranunculus ficaria* were dispersed over vast distances.

WINCHELL (58) 1924 a.o. called attention to the danger to plants of dust rains carrying pathogenic organisms.

BLACKTIN (7) 1934 studied the dispersal of light particles in the air as clouds of smoke. He wrote about the aeroplankton as follows; "The living components of the staubosphere [4]) will be bacteria, moulds, fermenting organisms etc, actually living, and spores and seeds not yet germinated.... They are exceedingly numerous and wide spread and are an almost invariable accompaniment of the other staubosphere components.

The lightest are the spores of lichens, mosses, fungi, ferns, algae, lycopodiaceae, and all vegetable cryptogams.... spores and probably the lighter dust seeds and winged seeds must be classed with the finest volcanic dust, which tends to keep circulating round the earth rather than settling".

He mentioned J. Baxendell and A. R. Yarwood who reported a thick yellow-ochre-like deposit of algae, which fell as dust at Southport, England in March 1930.

c. The influence of rain, hail and snow.

Not only dust-rain, but also rain, hail and snow are of great importance for the dispersal of plants. Many investigators found very few spores even a sterile air after a shower and a lower germ content of the air in the wet seasons.

It was rain-, well- and snow water that caused the first discovery of bacteria by VAN LEEUWENHOEK.

EHRENBERG repeatedly examined snowwater, raindrops and dew on the presence of infusoria without convincing results.

According to him the researches of his predecessors Gleichen, Bory de St. Vincent and Schultze, who reported positive results are not quite trustworthy.

It is strange, however, that LINDNER (27) 1899 cultured different infusoria from rain- and snow water with ease. He collected the water in a "pure" porcelain dish and examined it microscopically

[4]) word coined by Blacktin and meaning the "dust-sphere".

before culturing in hay-extract or broth. He found *Vorticella, Paramaecium, Cercomonas, Trichomonas* etc. and concluded to the necessity of examining rainwater in times of infectious diseases and the necessity of comparing the results.

Belli (3) 1901 examined hail-stones on agar and gelatin-plates. He found only few bacteria and concluded that the hygienic importance of these investigations is slight.

Puschkarew is amazed about the results of Lindner. His experiments upon protozoa in rainwater produced only a few species. He made objections against the sterility of the dishes used by Lindner.

We refer to Chapter V for meteorological literature.

Discussion.

A review of the literature shows that aeroplankton has been investigated from several angles and by different methods.

Microbiological investigation of the atmosphere has been carried out either from a general point of view (of which the work of Pasteur is certainly the prototype) or in the interest of hygiene, phytopathology, the study of allergic phenomena or entomology.

The authors either submitted the material to direct microscopic examination or cultured bacteria, fungi, yeast, and actinomycetes from it, which organisms were found up to remarkable altitudes and at different locations.

For the distribution of protozoa we have to rely solely upon the work of Puschkarew, who sampled air only at "street level".

The various results obtained by different investigators are as a rule not only due to location, but also to a more or less degree of sterility precautions used in collecting air samples.

Only a single investigator mentions the presence of algae in the atmosphere; Ehrenberg found algae in dust samples, Miquel in air samples collected on ground level, Meier and Lindbergh observed algae at high levels, but none of these persons demonstrated the presence of the living state of such organisms, capable of active development, in the atmosphere.

The possibility of distribution of light particles through the atmosphere has been much theorized upon while actual observations are rather scant. Ridley and Blacktin e.g. suppose an enormous extent of movement of the spores of algae, mosses and ferns in horizontal- and vertical direction.

The proof for this, however, has never been furnished and Ridley pointed out the incompleteness of our knowledge especially in respect to algae, mosses and ferns.

Nobody has tried, as far as we are aware, to *culture* these plants from atmospheric air at different levels.

The Leyden Botanical Laboratory is interested in these studies chiefly from the point of view of distribution of living beings.

Because it seemed important to perform preliminary examinations, the Standard Aeroscope with asbestos, sand and glass-wool as filtering material and Rettgers Aeroscope described by RUEHLE 1915 were used by us and air samples were collected from the roof of the Botanical Laboratory at Leyden 14.5 meters high — the aeroscopes being connected with an air-pump and gas-meter.

The same experiments were made from the tower room of a 30 meters high tower of the hunting lodge of the National Park "De Hoge Veluwe". This lodge is situated in the middle of woods and sands and free from the influence of towns [5]).

As both buildings were coated with algae and as, moreover, convection currents can never be excluded around buildings, the results obtained, while referred to in this paper occasionally, are not considered to be entirely trustworthy.

As the airplane has come to our aid in the study of this curious transit of living forms, it is possible to collect air samples from various levels excluding infection from buildings and other places.

As we particularly wanted to demonstrate the presence of organisms capable of active development "adhaesion" methods, whereby the particles are caught on some adhesive surface seemed excluded.

Because we wanted to culture the lower plants in pure inorganic solution and as much as possible free from bacteria and yeasts, an organic "adhaesion" substance could not be used.

None of the instruments described fully our purpose. We, therefore, had to devise a sampling apparatus which should sample;

1°. a known and

2°. a large amount of air in

3°. a short time, while the possibility of

4°. extraneous infection should be excluded, as far as feasible.

[5]) We wish to express our thanks to the Board of Directors of the National Park "De Hoge Veluwe" for its kind assistance in these experiments.

CHAPTER II.
Sampling Apparatus for Aeroplankton.

a. *Description of the apparatus.*

A sampling apparatus, in which air is filtered through absorbent materials, and which may be handled by simple methods from the cockpit of a plane was devised at the Botanical Laboratory and constructed at the Kamerlingh-Onnes Laboratory under the supervision of Dr. C. A. Crommelin [6]).

Five numbered brass compartments (Figure 1) fit snugly in a brass box (9.5 × 4.7 × 15.7 c.m.). Each compartment has a lid with a rim all around. This rim overlaps the walls of the box when the compartment is closed. The rim was made as it contributes to the sterility of the closure, because we know that spores do not seem to go "around corners" (principle of Pasteur).

Inside each compartment is a brass tube B, connected by means of a steel spring to a screwed clamp Sc — a tube (B T) surrounding the steel spring was made later to avoid jerking. This spring pulls the brass tube into the compartment. Pulling the handle by means of a wire may open the compartment until checked by a bar S.B. which acts as a stop. Inside the brass tube a glass tube is placed in which an air-absorbing mass (glass wool) is loosely stuffed (A). The mass is kept from sliding by a piece of gauze (G) clamped to the walls by means of a glass ring (R). If sand is used as absorbing mass, two pieces of gauze are used to keep the sand in place. The glass tube B cannot fall out of the brass tube, the bar S.B. being so adjusted that when the compartment is opened as far as possible the glass tube rests on the wall of the outer box.

As the action of this apparatus, when mounted under the wing of an airplane cannot be observed from the cockpit, an electric lamp installation (Phot. 1 Tab. III) [7]) was constructed as follows; A contact, which lights a lamp in the cockpit, is made by a piece of platinum soldered on the brass box (B) and a piece of platinum on a brass stirrup (S) fixed on the rim of a compartment, as soon as the handle sets a compartment in motion. The circuit is broken when a compartment is nearly full open. Then a second contact is closed by a piece of platinum on the bar S.B. (P) and on the bottom of a compartment (C). The circuit remains closed

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[6]) My best thanks are due to Dr. Crommelin for his valuable assistance.

[7]) The glass wool of the opened compartment was transferred to the other side of the tube by the photographer.

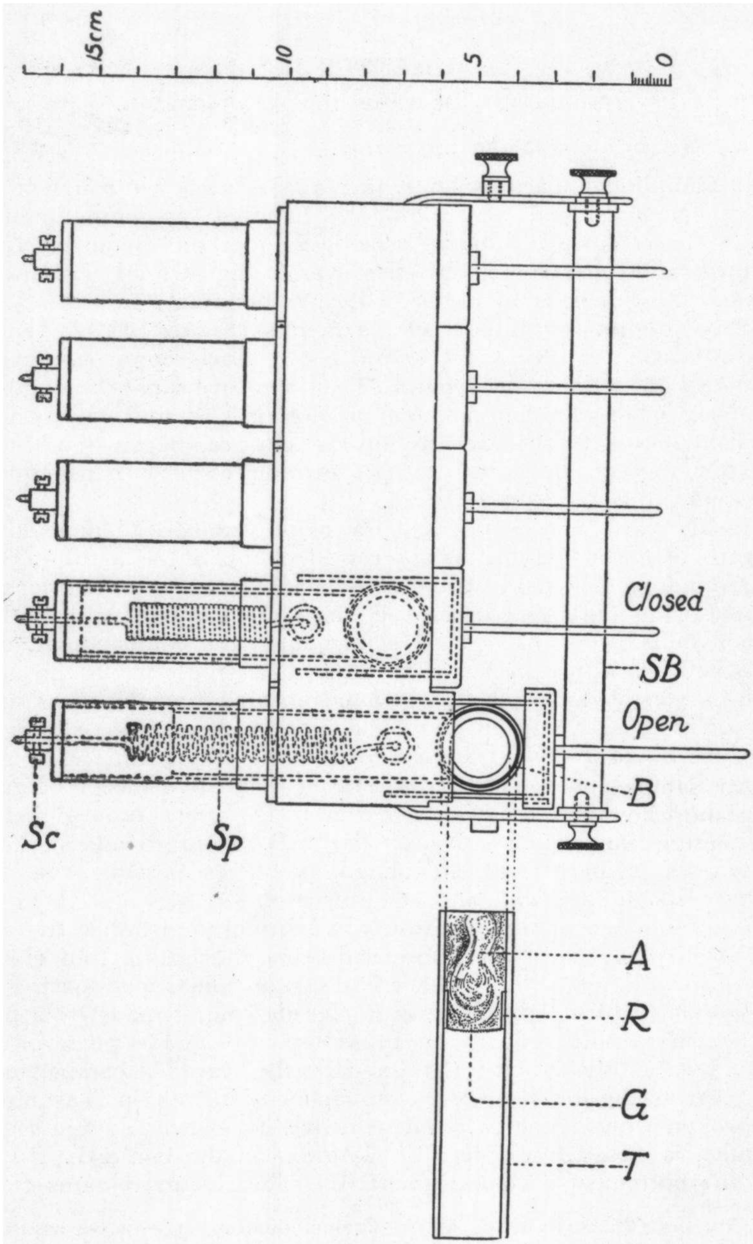

Figure 1.
Longitudinal section through sampling-apparatus. Further explanation in the text.

during the time a collection is made. Loosening the handle breaks the circuit, after that the stirrup closes the circuit again until the compartment is closed.

By means of these light-signals it is possible to control the complete opening and the entire closure of each compartment [8]).

b. *Absorbing substance and filtration-capacity.*

First of all the most suitable absorbent material was sought for. Through the kindness of the Direction of the Laboratory of Aerodynamics Delft, and Mr. G. Broersma, assistant at that Laboratory, the filtration-intensity of air through various absorbent materials was investigated in the wind tunnel in an air current of 35 M/sec.

The filtration-resistance in glass wool proved to be least of the three materials (asbestos, sand and glass wool) tested.

TABLE 1.

Nature of substances	Diameter of tube	Material spaced over	Amount of Material	Filtering capacity in Liter/minute
sand	15.2 mm	10 mm	—	12
asbestos fibre	15.2 „	30.5 „	500 mgr	37.5
Glass wool	15.2 „	32 „	500 „	77.5

Air samples taken from the roof of the laboratory and from a plane showed that glass wool and asbestos have a similar absorptive capacity for germs, but that fewer organisms were caught in a filter of 1 cm of sand as in 3 cm of glass wool.

Air samples taken from a plane 16-VI-'36 and 14-VII-'36 showed the following results (cfr. table 2).

One compartment was kept closed as a rule as control and remained sterile — 1 cc of water taken from the filter-mass, which was shaken with 15 cc of sterile water for 15 minutes, was used to infect broth-agar-plates.

Because of the slight filtration-resistance of the glass wool, but also because of the fact that asbestos fibre, when shaken with water, forms a cloudy suspension, which has to be filtered prior to culturing, we decided on glass wool as absorbing substance. Mr. Broersma, moreover, had the kindness to test the

[8]) I am much indebted to Mr. A. J. Stuivenberg, Master Mechanic of the Botanical Laboratory, for the construction of this installation and for his aid in mounting the apparatus on the airplane.

TABLE 2.

Date of flight	Compartment	Altitude in meters	Plane velocity in KM/hour	Collection time in minutes	Nature of substance	Material spaced over in mm	Amount of material in mgr	Colonies on broth agar
16-VI-'36	2	100	180	10	sand	10	—	3
	4	100	180	10	glass wool	32	500	27
	5	5100	125	12	glass wool	32	500	0
14-VII-'36	1	75	162	10	sand	10	—	57
	3	75	162	10	glass wool	32	500	107
	5	—	—	unexp.	glass wool	32	500	0

filtration capacity of a lenght of 32 mm of glass wool in a tube at various "tunnelspeed" (speed of air in windtunnel). Figure 2. shows the results of this experiment. Mr. Broersma states;

"With respect to the calibration of the flow through the tubes of the instrument only results of preliminary measurements made in the Laboratory for Aero- and Hydrodynamics at Delft on a single tube of approximately the same diameter and thickness but of a length which was a multiple of the length of those used in the instrument are given.

Characteristic of the results attained is the straight line in the diagram given as Figure 2. which approximately represents the connection between the flow through the tube as an interpolation of the measured points which are indicated separately.

It should be noted that when applying the different curves to the instrument interaction of the various tubes and other parts of the instruments as well as interference of the flow around the wing of the airplane to which the instrument is fixed with the flow around the instrument is not counted for.

The results derived from the measurements on the single tube should be taken as a rough approximation only giving an idea of the order of magnitude.

Moreover attention is drawn to the fact that the highest tunnelspeeds were of the order of 130—140 Kilometers per hour whereas the speed of the airplane is up to 170—180 Kilometers per hour. For the higher speeds it has been assumed for the present that the connection between the flow through the tube and the speed of the airplane remains linear.

In consideration of the facts mentioned it seems diserable to perform velocity measurements of the flow through the tubes

with the instrument fixed to the airplane in flight or at least to make measurements on the tubes in the instrument suspended in the windtunnel."

Contents of tube: 500 milligrammes of glass wool spread out over 32 millimeters. Temperature 13.9° C. Barometer 750.0 mm. Density 0,1236 KG M—4 sec.2 (Newton).

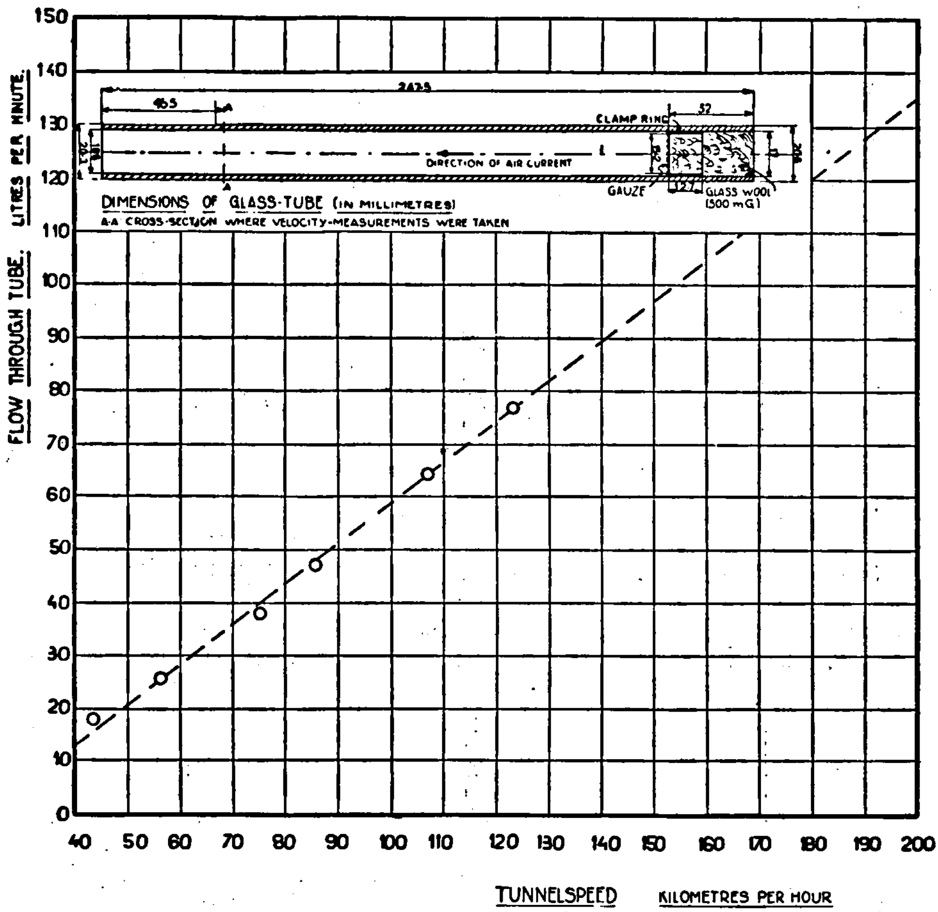

Figure 2.

c. *Control experiments in the windtunnel.*

The windtunnel was placed at our disposal for yet another

experiment. In order to ascertain whether the closed compartments of our apparatus remained sterile in an air current a concentrated suspension of spores of *Penicillium glaucum* was blown into the tunnel by means of a spray. The tubes were provided with glass wool, the instrument was mounted in the centre of the tunnel. Compartment 2 and 4 were opened, while 1, 3 and 5 remained closed.

In an air current of a velocity of 35 meters/second approximately one cubic meter was allowed to pass the filter mass, after which the mass was shaken with 15 cc of sterile water for 15 minutes. 1 cc of water was used to infect plates of malt-agar and broth-agar.

Results after two days incubation at 25° C:

TABLE 3.

Compartment	Colonies on malt-agar	Colonies on broth-agar
1	0	0
2	130	160
3	0	0
4	120	170
5	0	0

The unexposed compartments apparently remained sterile, while the exposed compartments showed from 1800—2500 viable colonies per cubic meter. The experiment was repeated with a yeast suspension, compartments 1,3 and 5 being opened this time.

TABLE 4.

Compartment	Colonies on malt-agar	Colonies on broth-agar
1	12	17
2	0	0
3	20	37
4	0	0
5	191	220

The contents of compartments 2 and 4 remained sterile, while compartments 1, 3 and 5 showed to be heavily infected.

d. *Mounting on the airplane; control flights.*

The former Commanding Officer of the Aviation Division at Soesterberg, General P. W. Best actively collaborated by detailing

Lt. E. Visch, Chief of the Meteorological Service and Lt. G. Oppenhuizen to collect the air samples and gather meteorological data at the time and place of sampling. We wish to thank these gentlemen and particularly Lt. Visch for their unfailing and enthousiastic help.

As an infection by the pilot had to be excluded as far as possible, the instrument was mounted by means of two clamps under the lower wing of a Type F.C.V. Rolls-Royce 450 H.P. biplane (Phot. 2 Tab. III) by the Technical Service at Soesterberg. This apparatus is mounted in such fashion that the axis of the glass tubes will be parallel to the axis of the plane. Five Bowden cables connect the clamps of the individual compartments with the cabin where they may be opened at the desired altitude.

The Bowden cables (Phot. 3 Tab. IV, C) pass through a plate (P), in which five holes are drilled, each hole being numbered. This plate is mounted on a wooden plate in the cockpit. Each wire may be placed into a groove (G) cut in the handle (H) and may be pulled along with the handle over a notched plate (N).

Battery (B) and lamp (L) for the light-signals are fastened on the same board.

Before the sampling experiments were started a control flight was made up to 5000 M. in order to ascertain whether the results would become unreliable by infection from the ground or from the plane.

As long as the plane is "taxi-ing" over the field, the apparatus is covered by means of a bag, which is removed when the plane is clear from the ground. While mounting to the desired altitude (5000 M) the apparatus and the plane are "washed" in an air-current.

Incubating filters from exposed compartments on organic media none or very few bacteria, yeasts or fungi could be raised from air sampled at 5000 meter altitude, while air sampled e.g. at 75 meter altitude during the same flight showed the presence of a copious number of organisms. One compartment was kept closed as control and remained sterile. 1 cc of water, taken from the filter-mass, which was shaken with 15 cc of sterile water for 15 minutes, was used to infect agar-plates.

The experiment was repeated three times with similar results, which is illustrated in the next table.

Organisms grown from air samples taken from a plane 18-V-'36; 16-VI-'36; 14-VII-'36.

TABLE 5.

Flight	Compartment	Altitude in meters	Plane velocity in KM/hour	Collection time in minutes	Colonies on broth-agar
18-V-'36	3	500	185	10	16
	4	5000	145	10	1
	5	—	—	unexposed	0
16-VI-'36	1	5100	125	12	0
	3	5100	125	12	0
	4	100	180	10	27
	5	5100	125	12	0
14-VII-'36	2	5000	207	12	3
	3	75	162	10	107
	4	5000	207	12	5
	5	—	—	unexposed	0

An Altigraph Record of Flight 14-VII-'36 is given in Figure 3.

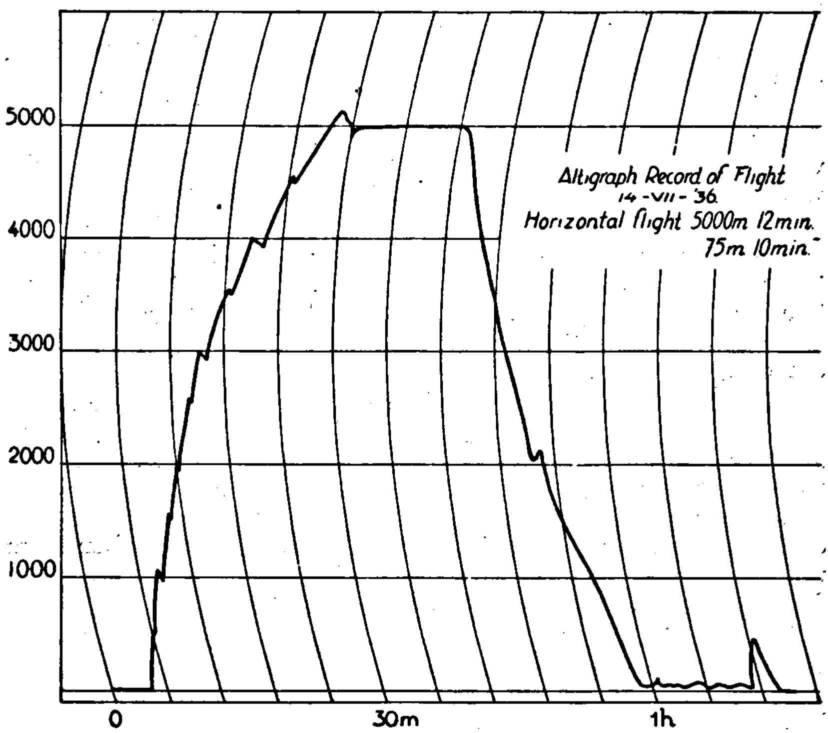

Figure 3.

e. *Sampling flights.*

The air samples are collected at about 2000, 1000, 500 and 100 meters altitude with an exposure time of 10 minutes, the pilot mounting first to the highest level, in the vicinity of Soesterberg and the Veluwe.

An Altigraph Record of each flight is made. Alternately one compartment was kept closed as control.

As the plane velocity varies from 150 K.M./hour to over 200 K.M./hour at different altitudes, ten minutes sampling is sufficient to filter about one cubic meter of air (see Figure 2).

The longer the collection time lasts so much the more air is filtered and more germs may be caught.

In view to this it seemed important to make the exposure time longer. This time was doubled in the later experiments, to begin with 16-III-'37.

A collecting flight was only made under favourable atmospheric conditions; fair weather and a strong dry wind.

But as our climate is humid and rainy occasionaly these flights had te be put off for a time or had to be made rather shortly after a period of rain.

The meteorological data at the time of each flight and three days before are noted by the Meteor. Service at Soesterberg. Temperature, Humidity, Pressure, H_2O-Vapour Pressure and Max. Vapour Pressure, Wind direction and -velocity are measured at different altitude, while the general weather observations as cloudiness, visibility, precipitation are mentioned during these three days. (See further Chapter V).

CHAPTER III.
Laboratory Procedure.

Before mounting the apparatus is sterilized in the autoclave for 30 minutes at 120° C.

a.. *Taking the samples from the apparatus.*

The instrument is returned as soon as possible to the laboratory after each collection-flight.

The glass wool has to be removed from the inner glass tube. This was done using aseptic methods in a culture chamber.

The bar of the apparatus is taken away in order that each compartment may be opened so far that the glass tube may be wholy removed from the instrument. Immediately after opening

the compartment the glass tube is closed at each end by two sterile cotton stoppers.

Then the glass wool is transferred to an Erlemeyer after pushing it out of the glass tube by means of one of the cotton stoppers.

The glass wool is shaken into the Erlemeyer containing 35 cc of sterile water during a period of 15 minutes.

After this the quantity of water is divided by means of sterile pipettes over six different inorganic culture solutions, using about 3 cc for each culture-bottle.

One cc of the water respectively is transferred to a petri-dish to which broth-agar- and to a petri-dish to which malt-agar is added.

A seventh solution is poured out over the glass wool and over the remainder of the water.

The content of the control compartment is treated in quite the same manner.

As this procedure cannot be performed by one person I was assisted by Miss J. de Zeeuw, assistant at the Botanical Institute [9]).

A slight variation in this procedure was made during the later experiments.

As it was thought possible that germs were not fully shaken out of the glass wool in using sterile water and as this might diminish their chance to find a specific culture medium, the glass wool was placed, instead of using an Erlemeyer with sterile water, into a sterile petri-dish and divided into nine, approximately equal, portions. This division was carried out by means of sterile forceps. The portions were transferred to seven different culture fluids.

Two of these nine parts were cultured with broth- and maltagar.

The loading of the apparatus is described in Chapter II.

b. *Culture solutions.*

The composition of the culture solutions used in these experiments is of the greatest importance.

As we wanted to demonstrate the presence of different organisms in the air samples, a selective method by using various nutrient fluids might be preferable.

The use of a great variety of culture media however presents difficulties, because the air samples ought to be diluted into as many portions whereby the chance of a germ to strike an un-

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[9]) At this place I wish to thank Miss J. de Zeeuw for her faithful help.

suitable milieu might be increased.

This idea was confirmed, moreover, by preliminary experiments, in which sixteen different solutions were used. The results did not give an indication as to the development of a certain species in its specific milieu.

We therefore confined ourselves to a few more general culture fluids of the following composition;

Solution I *for Algae, Mosses, Ferns.*
According to Pringsheim and used in the concentration mentioned by VON SCHELHORN (50);
1 g KNO_3
0,1 g $(NH_4)_2HPO_4$
0,05 g $MgSO_4$
0,002 g $CaCl_2$
0,002 g Fe_2Cl_6
1000 g aq. dist.
a pH of the solution is : 7
b pH : 4—5
c pH : 8

Solution II *for Salt-organisms.*
According to VAN NIEL;
3,6 % NaCl
0,02% K_2HPO_4
0,02% $MgCl_2$
0,02% KNO_3
0,1 % $NaHCO_3$
tapwater.
pH of the solution is 8—9

Solution III *for Protozoa.*
hay-extract [10])
pH : 7

The different pH's were obtained by addition of 0,1 n NaOH and 10% H_3PO_4 and determined by the indicators thymol blue, phenol red, brom-cresol purple and brom-cresol green.

The pH determination was performed before and after sterilization. A small change (—0,3), which appeared as a rule after

[10]) Various fluids were tested in preliminary experiments without definite results e.g.
broth of oxo and broth of horse-meat in different concentration,
hay-extract with- and without broth, a culture solution of Lwoff for Infusoria (cfr. KUFFERATH (24)).
As hay-extract is used in a great number of experiments on Protozoa, we concluded to this medium.

sterilization was noted but not altered. When larger differences occurred adjustment was made by the addition of a few drops of sterilized NaOH and H_3PO_4 solution by means of sterilized Pasteur pipettes. The pH's were controlled at regular intervals.

60 cc bottles of common glass were filled with 40 cc of culture medium.

As it is known that soil-extract stimulates growth, some cc were added to each bottle according to PRINGSHEIM (43) in proportion of 1 : 25.

Because it appeared from preliminary experiments that the incubation time of algae, mosses and ferns may go up till six months and longer, each bottle was closed by fastening a piece of tin-foil over the cotton-stopper to prevent infection.

The above mentioned solutions were tested by infecting Solution I (a, b and c) and Solution III with soil samples; Solution II with the salt organism *Dunaliella viridis*.

Different Algae e.g. *Desmidiaceae, Diatomeae, Chlorophyceae* and *Cyanophyceae* and *Protozoa* were found in Solution I and III after an incubation-time of about three weeks.

Solution II showed an even green colour, caused by *Dunaliella* after the same time.

The culture bottles were placed in a north window where the temperature fluctuated between 18°—24° C. One bottle containing Solution I b, and one culture bottle with Solution II were placed in a light-box at a constant temperature of 17° C.

Summarizing, the seven different culture methods are as follows;

1	Solution	I	pH : 4	t : 18—24° C.
2	„	I	pH : 7	t : 18—24° C.
3	„	I	pH : 7	t : 17°
4	„	I	pH : 8	t : 18—24° C.
5	„	II	pH : 8—9	t : 18—24° C.
6	„	II	pH : 8—9	t : 17°
7	„	III	pH : 7	t : 18—24° C.

Inorganic solution I and II were used to avoid the growth of bacteria and fungi.

The culture bottles were examined from the outside each week by means of a pocket-lens or microscope. As the development of the organisms may take six months and even longer,

the culture bottles were kept in culture for about eight months. Immediately after appearance and proper development of an organism it was identified and transferred if necessary to a more specific medium.

As to the mosses and ferns, these were transferred to a sterile solid medium of unglazed tile, which fitted into a petri-dish with solution I b.

The *Cyanophyceae* were placed either in a solution for Blue-greens according to Harder (cf. KUFFERATH (24)) or on a solid medium as described above with Harder's solution.

The *Chlorophyceae* were cultured in solution I b with- or without a solid medium and on agar plates made of 2% purified agar and solution I b.

CHAPTER IV.

Results.

a. *Results of air-samples taken by means of an airplane.*

Tables 6 to 12 contain the results from 6 flights, only the Altigraph Record of Experiment IV (Figure 4) 17-IX-'36 is given.

As the incubation-time of the organisms may last six months and longer (cfr. Chapter III), the results of the experiments taken; 16-III-37, 24-III-37, 31-III-37, 12-IV-37 are not complete up till now and may not be compared with experiment I-VII.

Experiment I. TABLE 6. date: 23-VII-'36.

Compartment	Altitude	Exposure time	Plane velocity	Filtered airmass	Culture solution			Organisms
	in meters	in minutes	in KM/hour	in Liters	number	pH	t°C	
4	2000	10	171	1130	I	4		*Chlorella vulgaris* Beijerinck
4	2000	10	171	1130	I	4		*Funaria hygrometrica* Sibth
3	1000	10	167	1100	—	—	—*	0
2	500	10	175	1150	I	7		*Chlorococcum spec.*
1	200	10	167	1100	I	7		*Chlorococcum spec.*
1	200	10	167	1100	I	7,8		*Chlorococcum spec.*
5 control		0			—	—	—*	0

*) negative in all media.

The Altigraph Record showed that these exposures were started in the inverse succession.

Experiment II. TABLE 7. date: 15-VIII-'36.

Compartment	Altitude	Exposure time	Plane velocity	Filtered airmass	Culture solution			Organisms
	in meters	in minutes	in KM/hour	in Liters	number	pH	t°C	
5	2000	10	169	1110	—	—	—*	0
4	1000	10	170	1120	—	—	—*	0
3	500	10	175	1150	I	7		*Pleurococcus vulgaris* Naegeli
3	500	10	175	1150	I	7		*Fern prothallus*
3	500	10	175	1150	I	4,4		*Funaria hygrometrica* Sibth
2	100	10	172	1135	—	—	—*	0
1 control		0			—	—	—*	0

*) negative in all media.

Experiment III. TABLE 8. date: 28-VIII-'36.

Compartment	Altitude	Exposure time	Plane velocity	Filtered airmass	Culture solution			Organisms
	in meters	in minutes	in KM/hour	in Liters	number	pH	t°C	
5	2000 +100	10	181	1210	I	7		*Pleurococcus vulgaris* Naegeli
"	"	"	"	"	I	7,8		*Chlorococcum spec.*
"	"	"	"	"	I	4,4		*Chlorococcum spec.*
"	"	"	"	"	III	7		*Chlorella vulgaris* Beijerinck
4	1000	10	179	1190	I	7		*Actinastrum spec.*
"	"	"	"	"	I	7,8		*Stichococcus minor* Naegeli
"	"	"	"	"	I	7	17	*Pleurococcus vulgaris* Naegeli
"	"	"	"	"	III	7		*Stichococcus bacillaris* Naegeli
3	500	10	176	1160	I	7,8		*Aphanocapsa spec.*
"	"	"	"	"	III	7		*Chlorococcum spec.*
"	"	"	"	"	III	7		*Phormidium luridum* Gom. fa. *nigrescens* Fremy
"	"	"	"	"	III	7		*Aphanocapsa spec.*
1	100	10	175	1150	I	7	17	*Chlorella vulgaris* ** Beijerinck
"	"	"	"	"	III	7		*Phormidium luridum* Gom. fa. *nigrescens* Fremy
2 control		0			—	—	—*	0

*) negative in all media.
**) Dr. M. Lefèvre states; "Tube pH 7: *Chlorella vulgaris* Beijerinck et probablement aussi *Chlorella ellipsoidea* Gerneck".

Compartment 5 has been opened by mistake a second time during 5 minutes at 100 M. altitude.

Experiment IV. TABLE 9. date: 17-IX-'36.

Compartment	Altitude	Exposure time	Plane velocity	Filtered airmass	Culture solution			Organisms
	in meters	in minutes	in KM/hour	in Liters	number	pH	t⁰C	
5	2000	10	173	1140	—	—	—*	0
4	1000	10	174	1145	—	—	—*	0
2	500	10	174	1145	I	7		*Chlorococcum spec.*
"	"	"	"	"	I	7,8		*Chlorococcum spec.*
"	"	"	"	"	I	7	17	*Chlorococcum spec.*
1	100	10	171	1130	III	7		*Phormidium luridum* Gom. fa. *nigrescens* Fremy
"	"	"	"	"	III	7		*Hormidium flaccidum* A. Braun
3 control		0			—	—	—*	0

*) negative in all media.

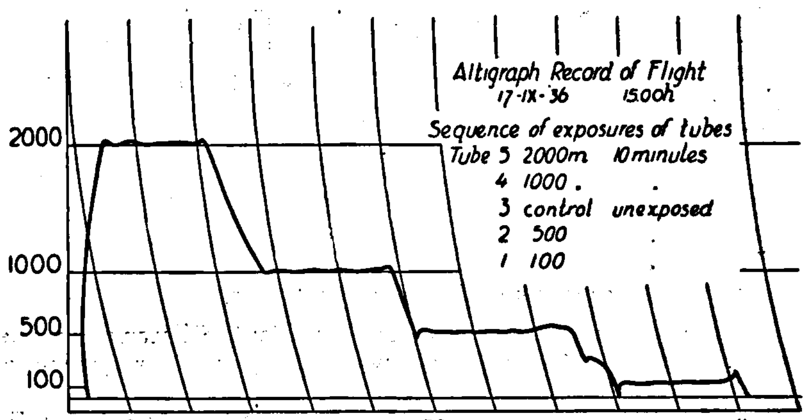

Figure 4. Altigraph Record of 17-IX-'36.

Experiment V. TABLE 10. date: 24-IX-'36.

Compartment	Altitude	Exposure time	Plane velocity	Filtered airmass	Culture solution			Organisms
	in meters	in minutes	in KM/hour	in Liters	number	pH	t°C	
5	2000	10	182	1215	—	—	—*	0
3	1000	10	178	1180	—	—	—*	0
2	500	10	169	1110	I	4,4		*Stichococcus bacillaris* Naegeli
1	100	10	171	1130	—	—	—*	0
4 control		0			—	—	—*	0

*) negative in all media.

Experiment VI. TABLE 11. date: 13-X-'36.

Compartment	Altitude	Exposure time	Plane velocity	Filtered airmass	Culture solution			
	in meters	in minutes	in KM/hour	in Liters	number	pH	t°C	
4	2000	10	180	1200	I	7		*Stichococcus bacillaris* Naegeli
3	1000	10	166	1095	—	—	—*	0
2	500	10	168	1 05	—	—	—*	0
1	100	10	160	1050	—	—	—*	0
5 control		0			—	—	—*	0

*) negative in all media.

Comparing experiments I-VII we find that;

I. In regard to the altitude an important difference in frequency occurs at various levels with the greatest frequency at 500 meters.

If we consider the exposure of compartment 5, experiment III as being made at an altitude of 100 meters we obtain the following graph.

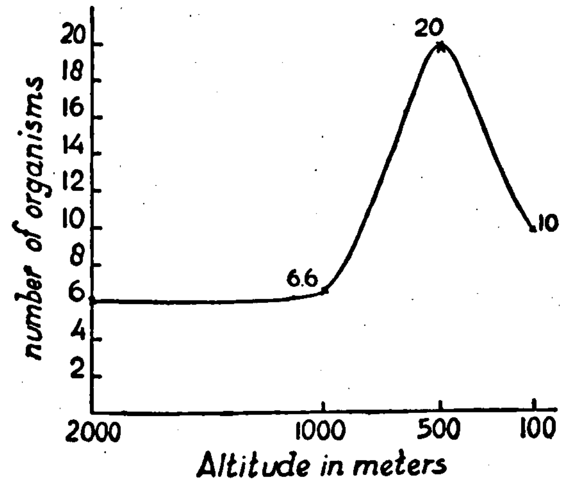

Figure 5.
Number of organisms obtained at various altitudes.

II. Concerning the development of organisms in the seven different culture fluids, the greatest frequency occurs in solution I, pH 7, as shows table 12.

Solution III originally used for Protozoa, appeared to be the most "fertile" culture fluid after solution I pH 7 and caused the

TABLE 12.

Culture solution	pH	Temperature in °C	Frequency
I	4	18—24	5
I	7	18—24	8
I	7	17	3
I	7.8	18—24	5
II	8.9	18—24	0
II	8.9	17	0
III	7	18—24	8

development of different green- and bluegreen Algae, while the salt solution did not yield any results up till now.

The following table shows the number of culture solutions containing organisms expressed in %.

TABLE 13.

Experiment	Culture solution Containing organisms in %
I	10.7
II	7.0
III	43.0
IV	14.3
V	3.5
VI	3.5

The highest percentage is found in experiment III (cfr. IV of this Chapter).

III. Of the organisms cultured we find the greatest frequency of *Protococcaceae* and *Chlorellaceae,* which is illustrated in the next table.

TABLE 14.

Class	Family	Species	Number of ** bottles containing species
Cyanophyceae	Chroococcaceae	*Aphanocapsa spec.*	2
"	Oscillatoriaceae	*Phormidium luridum* Gom. fa. *nigrescens* Fremy	3
Chlorophyceae	Protococcaceae	*Chlorococcum spec.*	9
"	Chlorellaceae	*Chlorella vulgaris* Beijerinck	3
"	Scenedesmaceae	*Actinastrum spec.*	1
"	Pleurococcaceae	*Pleurococcus vulgaris* Naegeli	3
"	Ulotrichaceae	*Stichococcus bacillaris* Naegeli	3
"	"	*Stichococcus minor* Naegeli	1
"	"	*Hormidium flaccidum* A. Braun	1
Musci	Funariaceae	*Funaria hygrometrica* Sibth	2
Filicinae *	?	?	1

*) The first leaflet on the fern prothallus has developed after 11 months but it cannot be classified as it is as yet too small.

**) = minimum number per 24 cubic Meters.

Figure 6 and 7 show the forms observed.

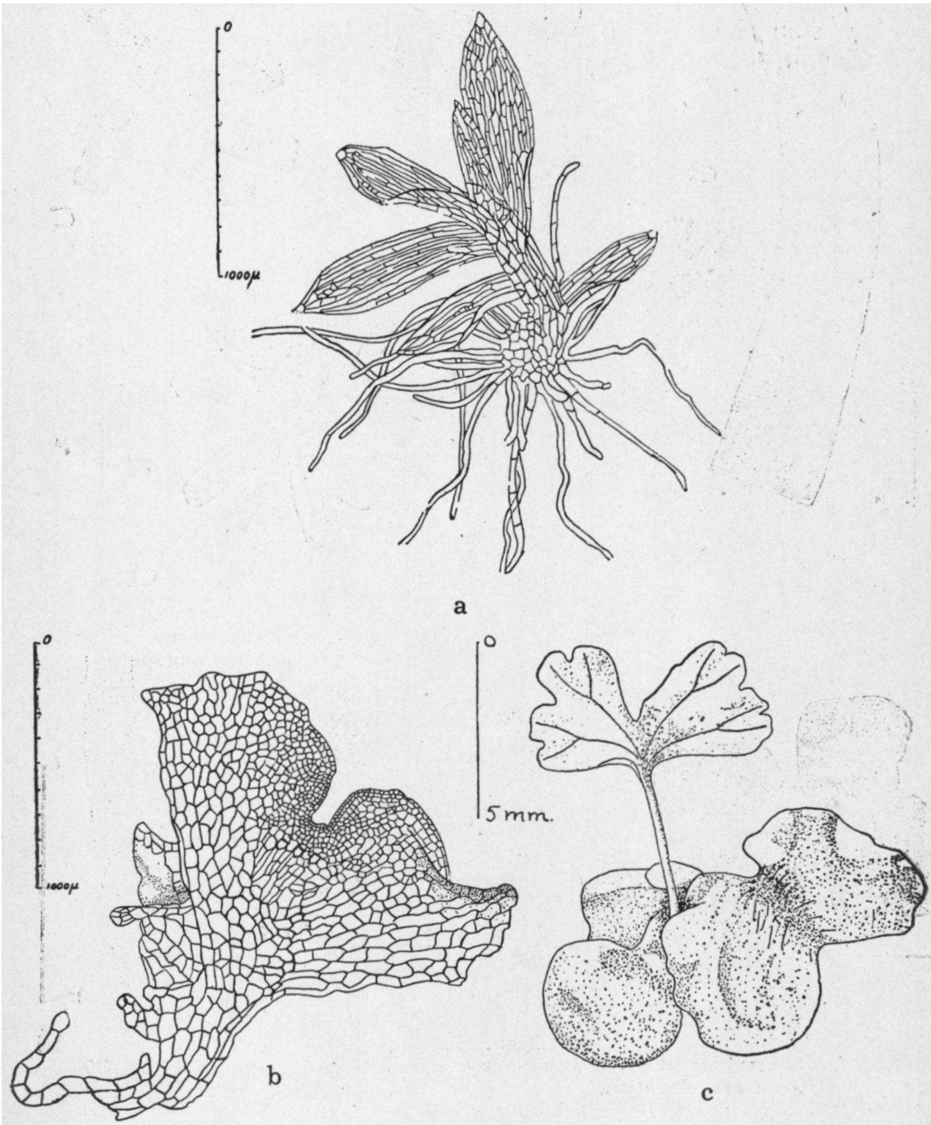

Figure 6 a. *Funaria hygrometrica* Sibth. grown from a spore sampled 23-VII-'36 at 2000 meters elevation. The microphotograph was inked and the silver dissolved with cyanide. b. Prothallus of fern, with spore still attached and three antheridial primordia, grown from a spore sampled 15-VIII-'36 at 500 meters elevation. Print prepared as under a. c. First leaftet of the fern. June 1937.

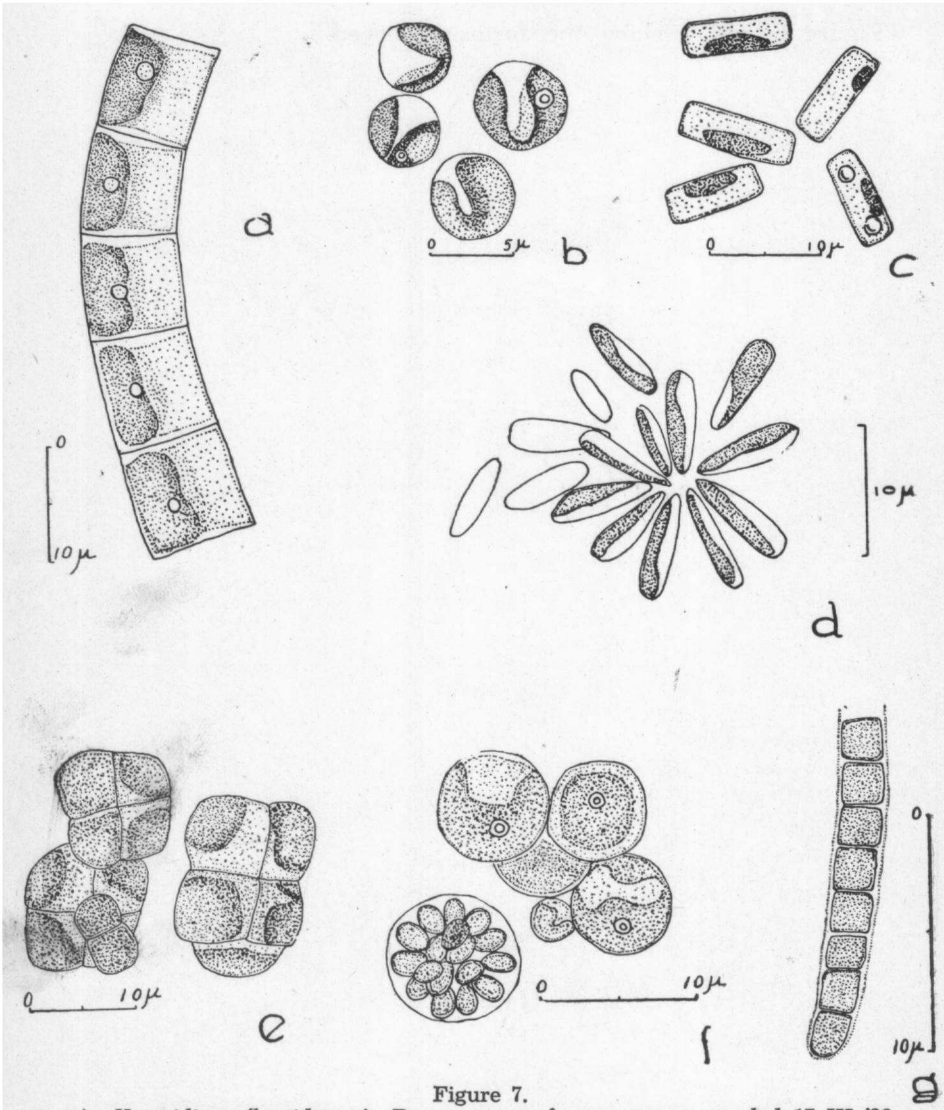

Figure 7.

a) *Hormidium flaccidum* A. Braun grown from a spore sampled 17-IX-'36 at 100 meters elevation.
b) *Chlorella vulgaris* Beijerinck. For details see text.
c) *Stichococcus bacillaris* Naegeli. For details see text.
d) *Actinastrum spec.* grown from a spore sampled 28-VIII-'36 at 1000 meters elevation.
e) *Pleurococcus vulgaris* Naegeli. For details see text.
f) *Chlorococcum spec.* For details see text.
g) *Phormidium luridum* Gom. fa. *nigrescens* Frémy. For details see text.

The number of organisms found does not represent the total number for the following reasons.

The first reason is the possibility that an organism may strike an unfavourable milieu and may not develop. This is the reason why the glass wool was divided into nine approximately equal portions in the later experiments, instead of using an Erlemeyer with sterile water (cfr. Chapter III).

A second reason is the fact that one organism might have developed out of two spores.

Attention should be drawn to the fact, however, that nearly always pure cultures of algae, mosses and fern were obtained with only a single species of fungi or bacteria.

For these reasons we confine ourselves to qualitative statements.

A lower frequency-limit may of course be stated in any case, which is illustrated in the last column of table 14.

For *Chlorococcum* and for *Stichococcus*, which occurred in almost all of the culture media, these numbers may approach to a true average—*Phormidium*, however, only developed in hay-extract (three times), while *Pleurococcus* developed (three times) only in Schelhorns solution at pH 7. The milieu of the

TABLE 15.

Organisms	Incubationtimes of subsequent samplings	Average *
Aphanocapsa spec.	30, 137	
Phormidium luridum Gom. fa. *nigrescens* Fremy	151, 137, 119	136
Chlorococcum spec.	20, 20, 29, 29, 37, 37, 137, 48, 48, ?	45
Chlorella vulgaris Beijerinck	38, ?	
Actinastrum spec.	37	
Pleurococcus vulgaris Naegeli	42, 37, 129	
Stichococcus bacillaris Naegeli	128, 42, 209	
Stichococcus minor Naegeli	22	
Hormidium flaccidum A. Braun	119	
Funaria hygrometrica Sibth	20, 23	
Fern prothallus	36	

*) only given when the data seem to warrant averageing.

latter species may be, therefore, more restricted and their probably frequency should be at least seven times as high as given in the table. The small number of data do not warrant any more conclusions.

IV. We find the greatest development of organisms in Experiment III (cfr. table 8 and 13). As this finding depends on meteorological factors, we refer to Chapter V.

V. The length of the incubation-time for the different organisms in shown in table 15.

The classification of the Algae was carried out with the aid of Pascher's "Die Süsswasserflora Deutschlands Österreichs und der Schweiz" (Heft 5, Chlorophyceae 2 by E. Lemmermann, Jos. Brunnthaler and A. Pascher, Heft 6 Chlorophyceae 3 by W. Heering, Heft 10 Bacillariales by H. von Schönfeldt, Heft 12 Cyanophyceae by L. Geitler and A. Pascher) and Rabenhorst's "Kryptogamen-Flora XIV", Cyanophyceae by L. Geitler.

The Oscillatoriaceae were classified by Abbé F. Fremy, Professeur de l'Institut Libre-Saint-Lô and a few Chlorophyceae were classified by Dr. M. Lefèvre, Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, Paris.

The Musci being in a vegetative state, were classified by Mr. W. H. Wachter and Dr. S. van Ooststroom of the National Herbarium at Leyden with the aid of Dixon's "The Student's Handbook of British Mosses" [11]).

As the results of this paper deal only with green organisms, no effort was made to identify the various Fungi and Bacteria.

b. *Results of preliminary experiments.*

For the sake of completeness the organisms obtained from the air-samples taken from the roof of the Botanical Laboratory at Leyden and taken from the tower of the Hunting-Lodge of the National Park "De Hoge Veluwe" are mentioned hereafter, but these results are not considered to be quite trustworthy as both buildings are coated with algae (cfr. Chapter I).

The following table shows a summary of organisms and their frequency found in 11 different experiments-5 at Laboratory-roof, 6 at Hoge Veluwe-tower, in which the average number of filtered air-masses amounted to about 1800 Liter. The filtering substance was shaken in sterile water and this water was divided over 16 various culture solutions.

11) We wish to thank Abbé F. Fremy, Dr. M. Lefèvre, Mr. W. H. Wachter and Dr. S. van Ooststroom for their kind assistance in this matter.

TABLE 16.

Class	Family	Species	Number of bottles containing species	
			Lab. roof.	Tower H.V.
Bacillarieae	Naviculoideae	*Navicula minuscula* Grünow	1	
Chlorophyceae	Protococcaceae	*Chlorococcum spec.*	19	1
„	Chlorellaceae	*Chlorella vulgaris* Beijerinck	7	
„	Pleurococcaceae	*Pleurococcus vulgaris* Naegeli	6	
„	Ulotrichaceae	*Stichococcus bacillaris* Naegeli	5	
„	„	*Stichococcus minor* Naegeli		2
„	„	*Hormidium flaccidum* A. Braun	1	
Musci	Dicranaceae	*Ceratodon purpureus* Brid.		1

Attention must be drawn to the fact that *Chlorococcum* was found in these experiments in salt-solutions from 0.6 mol. Na Cl to 2 mol. Na Cl, while these solutions always remained sterile in the airplane experiment. This fact may be due to a heavier infection, in which the probability for variation is increased.

c. *Results from rain-water-samples.*

Besides the above mentioned experiments rain-water was investigated on germ-content.

The rain-water was caught in a sterile flask by means of a sterilised funnel after a dry period of two days on the roof of the Laboratory. The contents of this flask were divided over 16 different culture solutions.

TABLE 17.

Class	Family	Species	Number of bottles containing species
Chlorophyceae	Protococcaceae	*Chlorococcum spec.*	1
„	Pleurococcaceae	*Pleurococcus vulgaris* Beijerinck	4
„	Ulotrichaceae	*Stichococcus bacillaris* Naegeli	4
„	„	*Stichococcus minor* Naegeli	1

Table 17 and 18 show the results of a rain-water-sample of 21 cc taken in this manner on the 13th of November 1936 and a sample of 200 cc taken on the 18th of November 1936.

TABLE 18.

Class	Family	Species	Number of bottles containing species
Mycetozoa	Physaraceae	*Physarum nutans* Pers.	1
Bacillarieae	Naviculoideae	*Navicula minuscula* Grünow	1
Chlorophyceae	Protococcaceae	*Chlorococcum spec.*	6
"	Chlorellaceae	*Chlorella vulgaris* Beijerinck	2
"	Ulotrichaceae	*Stichococcus bacillaris* Naegeli	1
"	"	*Stichococcus minor* Naegeli	7
"	"	*Hormidium flaccidum* A. Braun	2
Musci	Hypnaceae	*Brachythecium rutabulum* B & S	1

Culture of the organisms obtained.

Mycetozoa-plasmodium, moss-protonema and fern-prothallus were transferred to sterile unglazed tiles, which fitted into petri-dishes with culture solution Ib (cfr. Chapter III).

A single moss-protonema was placed on ground-peat with the same nutrient fluid. Both methods were satisfactory as the moss-protonema yielded fair sized moss plants (Figure 9a) which could be classified.

The Mycetozoa-plasmodium developed spores and was classified.

The fern-embryo is still growing in one of our glass-houses, but it cannot be classified as it only possesses one immature leaf (see note pg. 422).

CHAPTER V.

Comparison of biological data with atmospheric conditions.

The greater part of the investigators noticed a relation between the germ-content of the air and both the seasons and other meteorological factors.

Such relations are mentioned in detail by MIQUEL (35) 1883 (cfr. Chapter I), who compared the number of bacteria which the air contained at the "parc de Montsouris" to temperature, humidity, wind-direction and -velocity during three successive years.

In regard to temperature and humidity Miquel states;

"En général, le chiffre des bactéries, peu élevé en hiver, croît au printemps, reste haut en été et baisse rapidement à la fin de l'automne".

Concerning the wind-direction he found that the "purest" wind came from the south and the most "contaminated" wind from the north-east, as this wind had to pass the town and was laden with microbes.

The next table shows the average number of bacteria per cubic meter and per season, which he found during three years of observation at Montsouris.

TABLE 19.

Autumn	Winter	Spring	Summer	Average
121	53	70	92	84

The Autumn seems the most favourable season for the dispersal of germs.

SAÏTO (49 a, b, c and d) too associated his findings with temperature, humidity, rain fall and wind-direction. He found a maximum number of fungi in July and October, a minimum in March — the air being nearly without germs after rain and after snowfall.

MOLISCH (37) gives similar results. He cites Aitken, who found in 1 cc of air 3200 microbes after a period of rain, 130.000 microbes after dry weather and 1860.000 microbes in a room [12]).

PUSCHKAREW (45) too noticed a lower number of Protozoa in November, December and March; these months were very rainy.

TRILLAT and FOUASSIER (56) 1914 demonstated in their examination of rain and fog, that microbes may serve as condensation-nuclei and concluded that it might be possible to free the air from microbes in rooms and halls by means of freezing the air.

Of the investigators who sampled air at higher altitudes a

[12]) These results seem excessively high to us.

comparison of biological data with atmospheric conditions was given more in detail by FLEMMING (17), HAHN (20) and PROCTOR (44 a and b) (cfr. Chapter I).

Flemming found an injurious influence of solar radiation and the greatest number of organisms occurring under clouds.

Hahn noticed that the germ-content seemed to go parallel with humidity and found a lower number of organisms in winter.

Proctor pointed at the number of widely fluctuating variables, which influence the biological results and at the difficulty to make comparisons under such circumstances. Proctor states;

"There is possibly a slight indiciation of lower micro-organisms counts on flights made on days following precipitation.... No definite relation of micro-organisms to either wind direction or velocity is apparent".

The experiments made by us include only a period of four months, but a comparison of results of these six sampling-flights

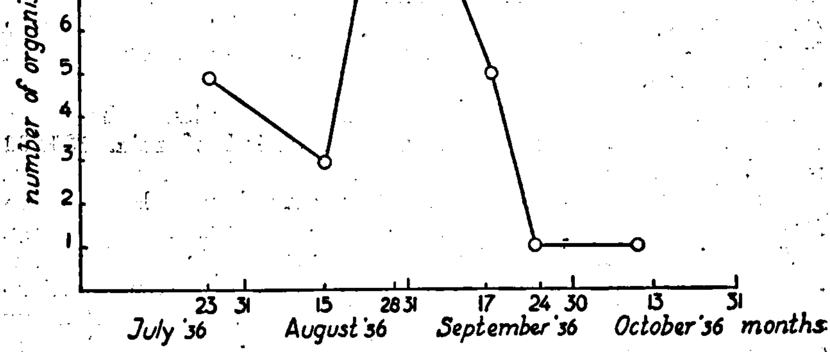

Figure 8.
Number of organisms obtained in subsequent sampling-flights.

to the six different sampling-data shows a variable number of organisms, which is illustrated in a graph (Figure 8).

The exposure of Compartment 5 (Experiment III) is considered as being taken at an altitude of 100 M. and the average number is calculated of the results of this exposure and of the exposure of compartment 1.

The meteorological data at the time of each flight and three days before the flight were gathered in the greatest possible detail by the Meteorological Service at Soesterberg (cfr. Chapter II e).

A brief survey and a comparison of the meteorological factors of these six periods were made by Lt. E. Visch [13]).

This table is given on pg. 432.

The detailed data pertaining to the sampling-flights number III and number VI follow;

III a Meteorological data 25—28 August '36, table 21.

TABLE 21.

Wind				Cloudiness		Precipitation
direction		velocity		kind	degree	m.m
sea-level	500-2000 M	sea-level	1000-2000 M.	St. St. Cu and Cu	$^3/_{10}$ $^6/_{10}$	0 m.m.
W.N.W. N.N.E.	W. N.N.E. E.N.E.	10 KM/h	30 KM/h	heavy clouds and scattered clouds		

a. *General remarks.*

According to the data of wind direction and-velocity represented in the weather-map, the air-masses have crossed the ocean during the greater part of these three days from a north-western and nothern direction of which 600 K.M. at sea-level and 1400 K..M on the levels between 1000—2000 M.

III b: Meteorological data on the day of flight; 28 August '36, table 22.

b. *General remarks.*

An extended area of high pressure was over N.W. Europe. The sky was clear and lightly clouded in our country and N.W. Germany.

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[13]) I wish to thank Lt. E. Visch for his invaluable help in this matter.

Summary of meteorological and biological data pertaining to sampling flights I—VII.

TABLE 20.

Period pertaining to sampling flight	Total number of organisms	Number of organisms at different altitudes	Weather situation			Track followed by air-masses	Wind direction
			Pressure	Cloudiness	Precipitation		
I. July 20-24	5	2000 M 2 1000 M 0 500 M 1 200 M 2	Surrounding area of low pressure	heavy clouds 23-VII heavy clouds	1—5 m.m. 23-VII o.m.m.	First part sea-track, last part land-track	W.N.W. S.
II. Aug. 12-16	3	2000 M 0 1000 M 0 500 M 3 100 M 0	Surrounding area of low pressure	heavy clouds and fog 15-VIII partly cloudy	1—5 m.m. 15-VIIIo.m.m.	First part sea-track, last part land-track	W.S.W. S.
III. Aug 25-29	11	2000 M - 1000 M 4 500 M 4 100 M 3	Extended area of *high* pressure	heavy clouds in the morning 28-IX *clear*	*0 m.m.*	First part sea-track, last part land-track	N.W. E.
IV. Sept 14-18	5	2000 M 0 1000 M 0 500 M 3 100 M 2	Decreasing area of high pressure	heavy clouds 17-IX heavy clouds above 3000 M	1—7 m.m. 17-IX 0 m.m.	First part sea-track, last part land-track	N.W. N.W.
V. Sept. 21-25	1	2000 M 0 1000 M 0 500 M 1 100 M 0	Decreasing area of high pressure	heavy clouds and fog in the morning 24-IX heavy clouds above 3000 M	± 1 m.m. 24-IX 0 m.m.	First part land-track last part land-track	S. E.
VI. Oct. 10-14	1	2000 M 1 1000 M 0 500 M 0 100 M 0	Surrounding area of low pressure	heavy clouds 13-X scattered clouds	1—5 m.m. 13-X 0.2 m.m. in the morning	First part sea-track, last part sea-track,	N.W. N.W.

TABLE 22.

Altitude in meters	Wind: KM/h Velocity	Wind: Direction	Temperature in °C	Rel. humidity in %	Pressure in m.m.H.g.	Cloudiness	Precipitation in m.m.	Horizontal Visibility
13*	4	S.	23.0	35	769.6			
100	8	S.E.	22.0	37	761.4	clear	0 m.m.	10—30KM
500	13	S.E.	18.8	39	727.6			
1000	16	E.S.E.	15.0	41	685.6			
2000	29	E.	10.3	29	609.0			50 KM

*) Altitude of Soesterberg in relation to Amsterdam mean level.

The air-masses crossed a land-track during the two days preceding the time of flight of which 240 K.M. from an eastern-north eastern direction at ground-level and ± 350 K.M. from an eastern direction on the levels between 500—2000 M.

Temperature and cloudiness point at a vertical aircurrent.

VIa. Meteorological data 10—13 October '36, table 23.

TABLE 23.

Wind: direction		Wind: velocity		Cloudiness: Kind	Cloudiness: degree	Precipitation	
						10-11 Oct.	0 m.m.
ground level	500-2000 M	ground level	500-2000 M	Cu and	5/10	11-12 „	1-3 „
N.E.	N.N.E.	7 KM/h	24 KM/h	Nb,Fr.Nb.	8/10 - 10/10	N.W.Germany	1-5 „
N.	N.W.	14 KM/h	32 KM/h	Partly cloudy			
N.W.				heavy clouding			
W.							

a. *General remarks.*

According to the data of wind-direction and -velocity represented in the weather map, the air-masses have crossed during these three days a track of 500 K.M. from a north-eastern direction (land-track) and from a north-western direction (sea-track). A sea-track was crossed on the levels between 500—2000 M of 1300 K.M. from a north-eastern and north-western direction.

VI b. Meteorological data on the day of flight; 13 Oct. '36, table 24.

TABLE 24.

Altitude in meters	Wind: Velocity KM/h	Wind: Direction	Temperature in °C	Rel. humidity in %	Pressure in m.m.H.g.	Cloudiness Kind	degree	Precipitation in m.m.	Horizontal Visibility
13	12	W.N.W.	14.5	57	760.2	clear		0.2 m.m.	
100	25	W.N.W.	13.6	91	752.0			in the	1—2 KM
500	50	W.N.W.	9.4	67	717.3			morning	
1000	36	W.N.W.	4.5	76	675.1	Cu	$^{8}/_{10}$		
2000	50	N.W.	-0.8	65	596.1	Ci			

b. *General remarks.*

High pressure in the S.W.-low pressure in the N.E. and N.W. Heavy clouds in the morning (drizzle). The air-masses crossed a sea-track during the 24 hours preceding the time of flight of which 300 K.M. from W.N.W. direction at sea-level and 1000 K.M. from a W.N.W. direction on the levels between 500—2000 M.

The favourable biological results of flight III are in agreement with the meteorological conditions. This is the reason why the wind-tracks of the air-masses sampled 28-VIII-'36 were reconstructed (Figure 9). The Figure shows that the land-wind of that day had been a sea-wind two days previously and had probably originated in western Norway, crossing the North-German plain.

From Lt. Visch' conclusion, why this period from 25—29 August differs from the others, we cite the following;

"Flight III which yielded the most favourable biological results was made during a period of fair weather (high pressure nucleus over our country) with clear — or partly clouded sky.

No precipitation fell in this period and, thirdly, ascending air-movement was possible because of the very slight cloudiness over an extended area, particularly in the last part of the land-track from eastern direction (see map of wind-tracks). In flight III it appeared, moreover, that the water-vapour content of the air up to the 2000 meter level was very low, while the temperature in this region was high.

The conditions under which these samples were taken differ in all these respects from the other cases in which precipitation occurred on — or immediately before, the day of sampling.

All the latter samples were taken in the neighbourhood of a

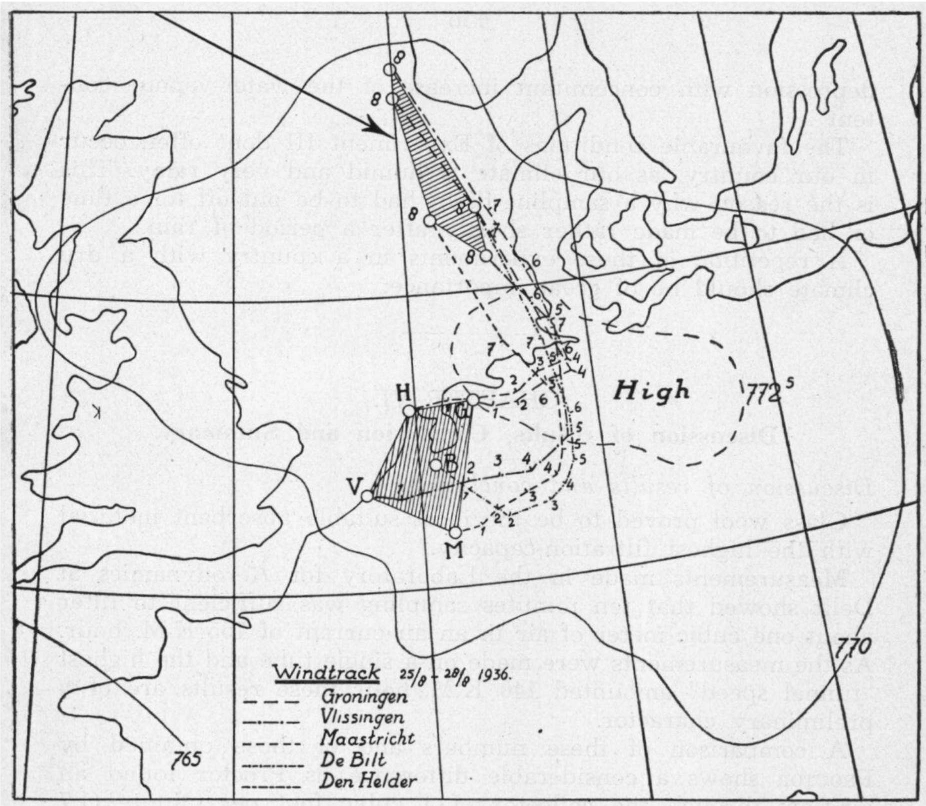

Figure 9.

Wind tracks 25-VIII—28-VIII-'36 as derived from meteorological data. Computed by E. Visch *).

1 Position of air masses 28-VIII 7.00 h.
2 " " " " 27-VIII 18.00 h.
3 " " " " 27-VIII 13.00 h.
4 " " " " 27-VIII 7.00 h.
5 " " " " 26-VIII 18.00 h.
6 " " " " 26-VIII 13.00 h.
7 " " " " 26-VIII 7.00 h.
8 " " " " 25-VIII 18.00 h.

The arrow S.W. of Norway indicates probable track previous to 25-VIII-'36. Five tracks are given ending in the meteorological stations:

H = den Helder
G = Groningen
B = de Bilt
V = Vlissingen
M = Maastricht

The quadrangle in the North-Sea indicates position of air masses on 25-VIII-'36.

The quadrangle formed by the Netherlands meteor.-stations H-G-M-V ludicates position of air-masses on 28-VIII-'36.

*) The methods used in this computation are treated by Visch (56).

depression with concomitant increase of the water-vapour content".

The favourable conditions of Experiment III dont often occur in our country, as our climate is humid and very rainy. This is the reason why a sampling-flight had to be put off for a time or had to be made rather shortly after a period of rain.

A repetition of these experiments in a country with a dry climate should be of great importance.

CHAPTER VI.

Discussion of results, Conclusion and Summary.

Discussion of results and conclusion.

Glass wool proved to be the most suitable absorbent material with the highest filtration-capacity.

Measurements made in the Laboratory for Aerodynamics at Delft showed that ten minutes sampling was sufficient to filter about one cubic meter of air in an air-current of 155 K.M./hour. As the measurements were made on a single tube and the highest "tunnel speed" amounted 140 K.M./hour these results are of a preliminary character.

A comparison of these numbers and of those obtained by PROCTOR shows a considerable difference as Proctor found an air-flow through the collector of 1 cubic foot per minute (1.7 cubic meter/hour).

Control-experiments in the wind tunnel convinced us that extraneous infection of closed compartments was excluded. This fact was confirmed, moreover, by the negative results of the controls, obtained in later experiments.

In order to ascertain whether the results would become unreliable by infection from the ground or from the plane, control-samples were taken up to 5000 M. with exposures at different altitudes. The samples were examined on bacteria and fungi and showed by the scarce quantity of organisms at the higher altitudes that these organisms came from the air at the levels indicated.

Proctor, as far as we are aware, is the only other author who claims similar certainty. He does not mention, however, how he obtained it.

The pilot, by mounting first to the highest altitude in control-flights up to 5000 meters, in sampling-flights up to 2000 meters

in order to air-wash the plane, kept alternatively one compartment closed as control.

Glass wool and inorganic culture solutions only showed a scarce development of bacteria and fungi.

As we cultured algae, mosses and ferns our findings cannot be compared with the results obtained by the other investigators working at higher levels mentioned in Chapter I, who used "adhaesion" methods and organic media as they chiefly wanted to demonstrate the presence of pathogenic organisms. "Plus occidit aer quam gladius" MIQUEL (35) cited from PRINGLE.

The air-samples were divided over seven different culture solutions of a "general" composition. The solution according to VON SCHELHORN pH : 7 produced the development of the greatest part of the organisms.

An objection against the use of general culture fluids is the fact that a more specialised organism cannot develop but a great number of different solutions presents difficulties as the air-sample has to be diluted over a similar number of portions whereby the chance of a germ to strike an unfavourable milieu might increase.

The incubation-time of algae, mosses and ferns may mount to six months and longer. This is the reason why the results of the four last sampling-fights could not be mentioned in this paper [14]).

The greatest frequency of organisms was found at 500 meters altitude. The fern-prothallus and one of the moss-protonemata developed from samples of the same altitude, the second moss-protonema was found in an air-sample obtained at 2000 meters altitude.

These fern and mosses represent the first species of these classes that have been cultured from air-samples collected in the troposphere.

The total number of organisms occurring in one cubic meter of air was not measured by our methods; thus far only qualitative work has been performed.

A lower frequency limit, however, could be ascertained. This shows that during a dry and warm period the air above the central part of the Netherlands may carry more than 7 cells of *Chlorococcum* per cubic meter.

Chlorococcum appeared to be the most frequent organism in these culture solutions. This fact is in agreement with the

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[14]) Up till now only one *Chloroccum* developed from these samples, which were all taken during periods of depression.

remarks made by Miquel 1883, who examines the air-samples on street-level microscopically and reaches the following conclusion:

"Il est tout à fait exceptionnel de rencontrer dans l'air de nos régions ces algues élégantes et rigides connues sous les noms de diatomées, de desmidiées et en général les représentants des végétaux vivants en eau profonde; par contre, les protococcus et les chlorococcus, que l'on voit envahir les toits des maisons, les murs et la terre humide, sont remarquablement plus fréquents en toute saison".

The number of organisms found in different sampling flights depends upon meteorological factors.

It is clear, however, that content and quantity of the aeroplankton is subject to a number of widely fluctuating physical factors not only of the environment and altitudes above the environment where samplingflights were made, but also of the countries from which the air-masses emigrated during the previous days.

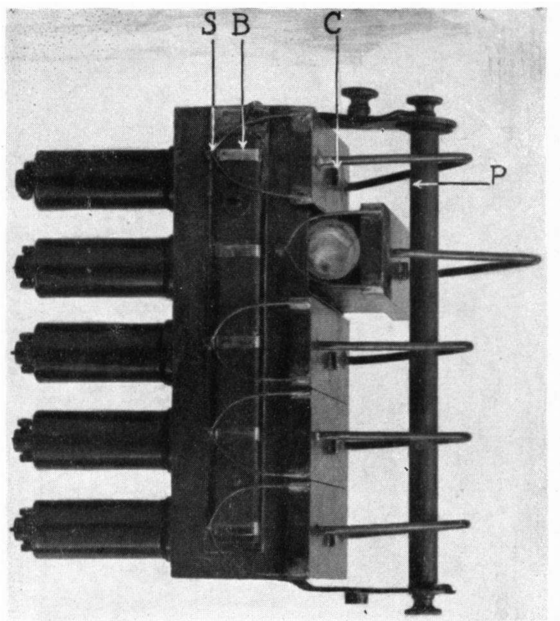
The "wind tracks" were reconstructed of the air-masses sampled 28-VIII-'36. It appeared from this reconstruction that the land-wind of that date had been a sea-wind two days previously and probably originated in western Norway, crossing the North-German plain.

A detailed investigation on the origin of the air-masses by means of their falling velocity in relation to meteorological factors should be of great interest.

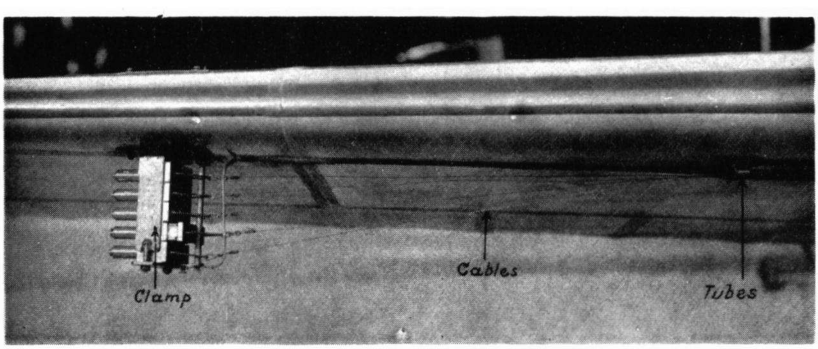
The air-masses filtered in our experiments are very small. Other investigations in which larger masses are filtered should increase the possibility of the development of many more organisms.

As the character of the organism obtained depends entirely upon the nature of the nutrient medium employed, the possibility is not excluded that organisms which failed to occur in our culture solutions, may develop in culture media of an other composition.

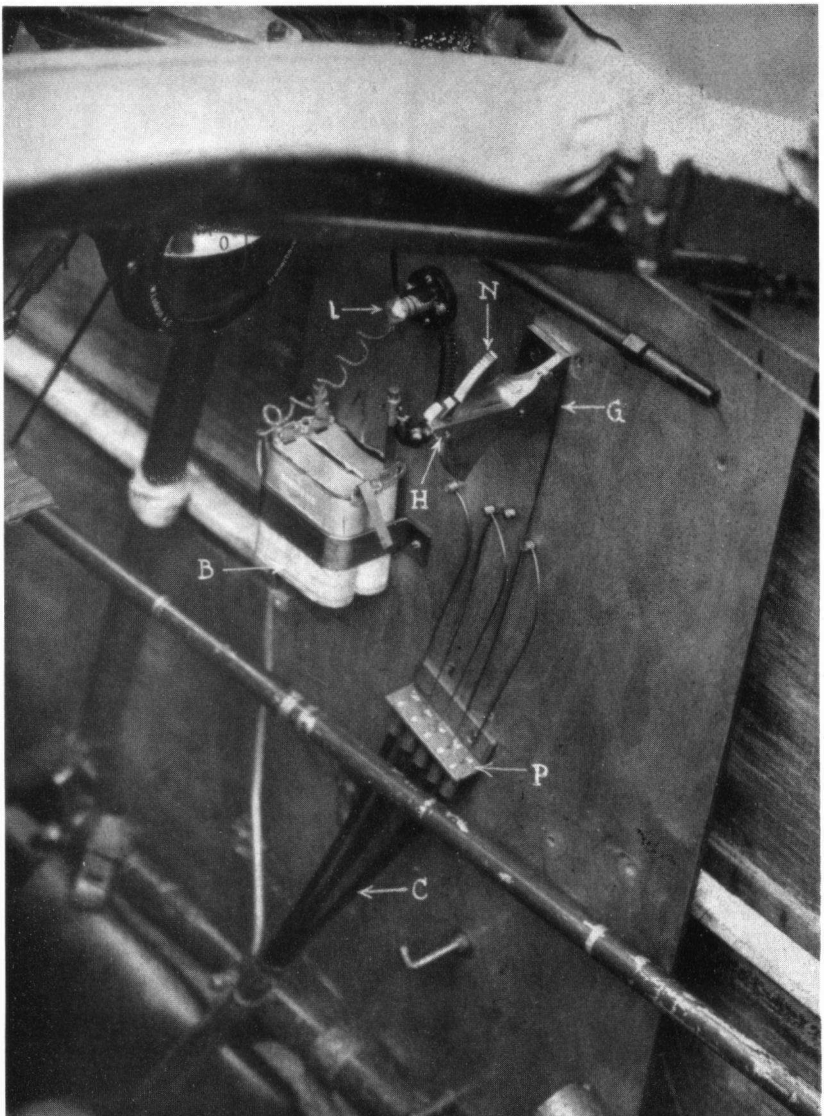
The results of the air samples taken from buildings and the results of the rain-water-samples showed similar organisms -*Chlorococcum* being the most frequent organism in these experiments too.



Photograph 1, by Phototechnical Service, Soesterberg. Sampling-apparatus with controls and one compartment opened. Further explanation in the text.



Photograph 2, by Phototechnical Service, Soesterberg. Sampling-apparatus mounted under the lower wing of the plane. One compartment opened.



Photograph 3, by Phototechnical Service, Soesterberg. Instrument-board in the observer's cockpit. For further details see text.

Summary.

1. A sampling-apparatus for aeroplankton was constructed and the exclusion of extraneous infection was ascertained by control-experiments.

2. The air-samples (in which about 1 cubic meter of air was filtered) from six airplane-flights during which 24 collections were made at different altitudes between 100—2000 meters, were examined and demonstrated the presence and active development of nine species of Algae, one species of Mosses and one species of Ferns.

3. The organisms appeared with different frequency-*Chlorococcum* being the most frequent.

4. The quantity of these organisms decreased at higher levels while repeatedly an appreciable number was found at 500 meters.

5. Meteorological factors exerted influence on the frequency of these organisms as a strikingly large amount of organisms was found after a noticeable dry period.

6. Air-samples taken from buildings by means of an air-pump at altitudes of 14.5- and 30 meters and rain-water-samples showed similar organisms. A slime-mould and a diatom were observed in these lower levels.

At this place I wish to express my most grateful thanks to Prof. Dr. L. G. M. Baas Becking for his invaluable advice and enthousiastic help during the time I worked at the Botanical Institute at Leyden.

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